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CONSTITUENTS OF HUMAN SMEGMA*

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Received January 25, 1960

Introduction

Smegma, a thick cheesy secretion, which accumulates between the inner lining of prepuce and the glans penis, has been associated with the production of cancer of penis. The incidence of cancer of penis in India is appreciably high in the South[1–2] compared to its incidence in Great Britain[3] and United States.[3] A primary reason for the high incidence of the disease has been attributed to the state of non-circumcision existing among the male population. This view is supported by the fact that clinically and statistically the incidence of cancer of penis is low in circumcised communities like the Jews[4–6] and Mohammedans.[4, 7, 8] An indirect evidence of the prophylactic value of circumcision is also presented by the statistical data that cancer of cervix uteri is more frequently encountered in women of uncircumcised communities.[7, 9, 10] An opinion contrary to the above views has however been expressed lately.[11]

Biological studies regarding the carcinogenic potency of human smegma, first initiated by Fishman and co-workers[12] are still under active inquiry[13] and our knowledge of the chemical composition[14, 15] of human smegma is still incomplete. The isolation of certain crystalline compounds from human smegma was reported[16] by us and details of the work are given in the present paper.

Results

Human smegma collected for experimental work was a heterogeneous material consisting of lipids, inorganic matter and debris. Different samples of smegma were therefore analysed separately. On an average, the total organic extractives were about one-fourth of the dry weight of the material. About two-thirds of these were petroleum-ether soluble. Petroleum ether extract thus formed the principal extract of smegma. By chromatographic fractionation of the extract, three residues were obtained as major constituents, which in their crude states gave the Liebermann Burchard test for

* The work was submitted for M.Sc. Thesis of the Bombay University in 1956.

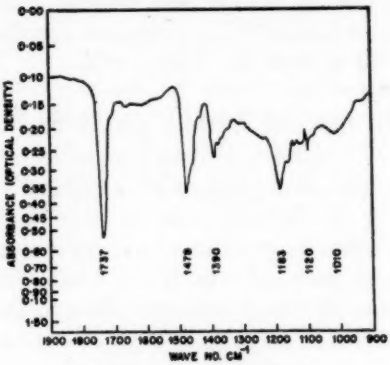
unsaturated sterols. Purification of these residues by repeated chromatography and crystallisation eliminated the adhering impurities and yielded three compounds—A, m.p. 79·8–80·6°; B, m.p. 123·8–124·4° and C, m.p. 139·6–141·2°.

Compound C.—Properties of compound C indicated it to be β-cholestanol. An authentic sample of β-cholestanol was therefore prepared by the oxidation[17] of cholesterol dibromide[18] to Δ^4-cholestene-3-one and subsequent reduction[19] of the ketone to β-cholestanol. A mixed melting point of compound C with β-cholestanol as well as of their acetates and benzoates remained undepressed. The identity was further confirmed by comparison of infra-red spectrum of compound C with that of β-cholestanol reported in literature.[20]

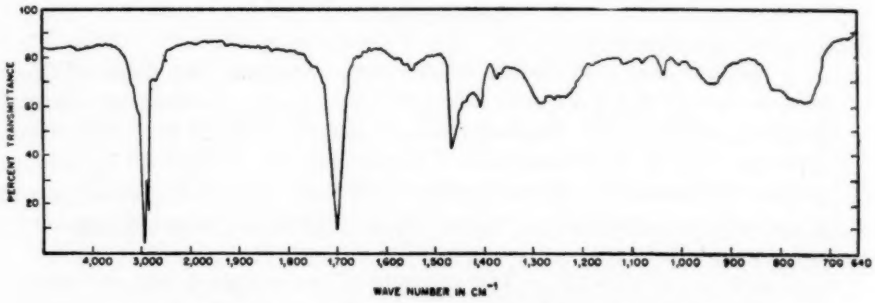
Compound A.—It was readily eluted from the chromatographic column by petroleum ether. It did not give unsaturation tests. An infra-red spectrum of compound A (*see* Fig. 1 *a*) showed a ketonic absorption band at 1737 $cm.^{-1}$ and the absence of ethylenic bond in the molecule. Hydrolyis of the compound with sodium ethoxide yielded a neutral product, m.p. 140·5° and an acid, m.p. 56·8–57·4°. The neutral product formed an acetate. Carbon-hydrogen analysis of the neutral product suggested for it the empirical formula $C_{27}H_{48}O$. A mixed melting point of the neutral product with β-cholestanol as well as of their acetates remained undepressed. This proved the neutral product to be β-cholestanol and compound A, an ester of β-cholestanol.

In an attempt to identify the acid moiety, recourse was had, in the initial stages of the work, to the syntheses of the esters of β-cholestanol with saturated fatty acids that could possibly be the components of the conjugate. Analytical data suggested for compound A, possible molecular formulæ (1) $C_{41}H_{74}O_2$, (2) $C_{43}H_{78}O_2$ and (3) $C_{45}H_{82}O_2$. Since β-cholestanol formed an alcoholic part of the ester and accounted for $C_{27}H_{48}O$, either myristic, palmitic or stearic acid fitted in with the acid part of the molecule. The possibility of compound A being β-cholestanyl stearate was excluded by the higher melting point of the latter. Cholestanyl myristate and palmitate, not recorded in literature so far, were synthesised, but were found to possess melting points higher than that of compound A.

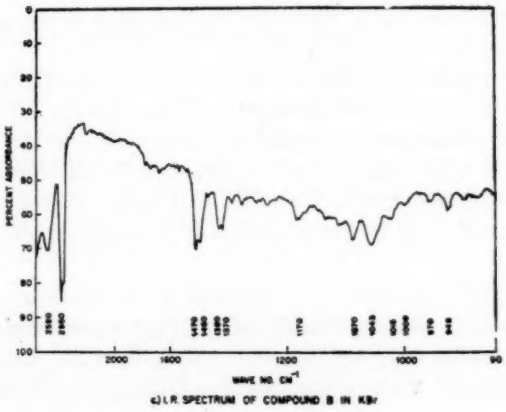
With the availability of further quantities of smegma, separation of the acid, m.p. 56·8–57·4, from compound A was possible. Neutralisation equivalent and analytical data of the acid indicated for it a molecular formula $C_{20}H_{40}O_2$. The possibility of the acid being arachidic acid, a saturated straight chain C_{20} acid was ruled out by the higher melting point of the latter.[21]



a) I.R. SPECTRUM OF COMPOUND A IN CCl_4



b) I.R. SPECTRUM IN CCl_4 OF ACID OBTAINED BY HYDROLYSIS OF COMPOUND A



c) I.R. SPECTRUM OF COMPOUND B IN KBr

FIG. 1

A comparison of the infra-red spectrum of the acid (Fig. 1 *b*) with those of saturated branched chain acids[22-23] reported in literature suggested that it might very probably be a branched chain acid.

Compound B.—On repeated chromatography and crystallisation, compound B melted at 124°. Analytical data have indicated for it an empirical formula of $C_{35}H_{60}O$. The I.R. spectrum of compound B was taken in both CCl_4 and KBr (Fig. 1 *c*). In both cases it shows a peak absorption at 3550 cm.$^{-1}$ This establishes the presence of a hydroxyal group (*s*) and is confirmed by the absorption peak 1043 cm.$^{-1}$, and positive antimony trichloride reaction.[24] The absence of peaks between 1780 and 1610 cm.$^{-1}$ (Fig. 1 *c*) eliminates any possibilities of carbonyl groups of a saturated or conjugated nature. From the evidence available, it is difficult to assign a constitution to the compound.

EXPERIMENTAL

Collection and preservation of Smegma.—Smegma was collected from patients attending (1) Veneral Diseases Department, Government General Hospital, Madras; (2) Medical College, Baroda and (3) the Out-Patients Departments from local hospitals. The material was scraped off by a blunt spatula and stored in the refrigerator in a wide mouthed glass-stoppered bottle under thiophene-free benzene until it formed a sizeable amount.

Extraction of Smegma.—The contents of the sample bottle, on receipt, were transferred to a tared flask; benzene was removed under reduced pressure, and the residue dried over calcium-chloride in a vaccum desiccator for a few days and weighed. The total quantity of the dried material obtained in six different lots was 94 g. Each lot was worked separately.

The dried material was exhaustively extracted with (1) petroleum ether (b.p. 60–80°), (2) benzene, (3) chloroform and (4) ethanol. The total organic solvent extractives of smegma amounted to between 22·7 to 30·5% (petroleum ether extractives 17–20, benzene extractives 2–2·5, chloroform extractives 0·7–1, and ethanol extractives 3–7%) of the dry weight of the material. The solvent insoluble residue, on incinerating at 600° for 4 hours, yielded 8–9% ash.

Separation and identification of compounds in the petroleum ether extract: General Methods.—(1) Melting points of purified compounds were taken on standard thermometers. (2) Specific rotations were determined with a 0·5 decimeter micro-cuvette in chloroform solution on a Triple field Hilger

micro-optic polarimeter. (3) The I.R. spectra* of compounds were measured in carbon tetrachloride or potassium bromide.

Chromatography.—A 10% solution of the petroleum ether extractives in petroleum ether (25 c.c.) was passed through a column (40×1·8 cm.) of activated alumina (75 g.) (Merck, Analytical Grade) and the chromatogram developed with petroleum ether. Elution of the bands was followed with the aid of ultraviolet light. The following fractions were collected, by stepwise elution.

Fraction	Eluted with	Residue on removal of the eluate
I	Petroleum ether (450 c.c.)	A—Colourless waxy residue
II	Benzene (350 c.c.)	B—Colourless residue
III	Benzene-ethanol (90: 10) (300 c.c.)	C—Pale yellowish white residue
IV	Ethanol	D—Yellow liquid

The crude residues A, B and C gave positive Liebermann Burchard test.

Residue A, on repeated crystallisations from acetone, yielded compound (A), m.p. 79·8–80·6 (Found: C, 82·4; H, 12·5%. Calculated for $C_{47}H_{86}O_2$: C, 82·6; H, 12·69%). Compound (A) possesses a waxy character. It is sparingly soluble in cold acetone, alcohol or alkali; and its solution in carbon tetrachloride does not decolourise a 2% solution of bromine in carbon tetrachloride.

Crystallisation of the residue (B) from dilute acetone yielded a crude compound (B), m.p. 117°, which exhibited a yellowish green fluorescence on the column but not in the eluate. Chromatographic purification of the compound followed by several crystallisations eliminated the adherent impurity and raised the melting point of the compound to 123·8–124·4°. It crystallised from acetone-water (65: 30) mixture in fine needles. Yield 0·6–0·7% of the dry weight of extractives. It gave wheat yellow colour and bluish-violet fluorescence when submitted to $SbCl_3$ reaction.[24]

* We are grateful to Dr. M. Kasha, Department of Chemistry, Florida State University, Tallahase, Florida, U.S.A., Dr. Rosenkrantz, Ph.D., Worcester Foundation for Experimental Biology, Mass, U.S.A., and Dr. K. S. Korgaonkar, Ph.D., Indian Cancer Research Centre, Bombay, for the recording and interpretation of I.R. spectrographs.

Hydrolysis of compound (A) and separation of β-cholestanol and acid.—The compound (A) (0·150 g.) in 1% sodium ethoxide solution (45 c.c.) was refluxed on a water-bath for 2 hours. From the reaction mixture, alcohol was removed and the residue after dilution with water was extracted with ether. The aqueous layer was tapped off and the ethereal layer was washed with water until the washings were neutral to litmus and dried over anhydrous sodium sulphate. On removal of ether, a neutral product was obtained which crystallised from methanol in shining plates. Yield 0·085 g.; m.p. 139·6–141·2° (Found: C, 83·0; H, 12·5%. Calculated for $C_{27}H_{48}O$: C, 83·45, H, 12·5%) $[\alpha]_D^{25°}$ $CHCL_3$; + 9·3. A mixed melting point of the neutral product with an authentic sample of β-cholestanol remained undepressed.

Acetate of the neutral product was prepared in the usual way by boiling with acetic anhydride and pyridine. It crystallised from dilute ethanol in shining plates, m.p. 110°. The product when mixed with β-cholestanol acetate did not show depression in melting point.

The alkaline hydrolysate and the washings were acidified with dilute hydrochloric acid and extracted with ether. The ether extract was washed with water until the washings were neutral to litmus and dried over anhydrous sodium sulphate. Removal of ether yielded an acid which crystallised from 60% ethanol. Yield 0·035 g.; m.p. 56·8–57·4° (Found: C, 76·9; H, 12·9%; $C_{20}H_{40}O_2$ requires C, 76·86; H, 12·9%). Neutralisation Equivalent (Found: 316·8; $C_{20}H_{40}O_2$ requires 312).

Purification of residue C.—The residue C was crystallised once from methanol. The crystalline product was dissolved in petroleum ether; the solution rechromatographed on alumina and the product eluted with benzene-chloroform (70: 30). It was then benzoylated and the benzoate was subjected to Liebermann Burchard reaction[25] and the recovered product saponified with alcoholic NaOH. Extraction of the unsaponifiable matter with ether and removal of ether from the extract yielded compound (C) which crystallised from methanol in shining plates. m.p. 139·6–41·2 $[\alpha]_D^{24°}$ $CHCl_3$; + 21·58°. Yield 2·3–2·5% (Found: C, 83·8; H, 12·24%. Calculated for $C_{27}H_{48}O$: C, 83·45; H, 12·4%). The compound showed no depression in mixed melting point when admixed with an authentic sample of β-cholestanol.

Acetate of compound (C).—It was prepared in the usual way. It crystallised from dilute ethanol in shining plates. m.p. 110° $[\alpha]_D^{23°}$ $CHCl_3$; + 11·3°. (Found: C, 81·1; H, 11·5%. Calculated for $C_{29}H_{50}O_2$: C, 80·7; H, 11·7%). A mixed melting point of the compound with β-cholestanol acetate remained undepressed.

Benzoate of compound (C).—Crystallised from dilute alcohol, m.p. 136·5° (Found: C, 82·8; H, 11·0%. Calculated for $C_{34}H_{52}O_2$: C, 82·87; H, 10·64%). A mixed melting point of the benzoate with β-cholestanol benzoate remained undepressed.

Preparation of β-cholestanol esters.—β-cholestanol palmitate, myristate and laurate were prepared by condensation of β-cholestanol with the corresponding acid chlorides, after the method of Fieser and Schneider.[26] The properties of the esters are as under:

(*a*) *β-cholestanol palmitate.*—Crystallised from acetone in colourless plates, m.p. 94·8–95·2. $[\alpha]_D^{22°}$ $CHCl_3$; + 10·9°. (Found: C, 82·0; H, 12·2%. Calculated for $C_{43}H_{78}O_2$: C, 82·36; H, 12·54%).

(*b*) *β-cholestanol myristate.*—Crystallised from acetone in plates, m.p. 91–91·2° $[\alpha]_D^{22°}$ $CHCl_3$; + 7·6°. (Found: C, 82·2; H, 12·6%. Calculated for $C_{41}H_{74}O_2$: C, 82·24; H, 12·50%).

(*c*) *β-cholestanol laurate.*—Crystallised from acetone in plates, m.p. 86–86·4° $[\alpha]_D^{24°}$ $CHCl_3$; + 9·8°. (Found: C, 82·5; H, 11·9%. Calculated for $C_{39}H_{70}O_2$, C, 82·04; H, 12·36%).

Summary

1. Three crystalline compounds A, B and C have been isolated from human smegma.

2. Chemistry of compound A has been studied in detail. It has been shown to be an ester of β-cholestanol with a saturated fatty acid of the formula $C_{20}H_{40}O_2$. Compound C has been identified with an authentic sample of β-cholestanol by mixed melting point and infra-red spectrum. Compound B occurs in very small amounts. Available evidence indicates it to be a hydroxy steroid.

3. Three new esters of β-cholestanol were prepared in connection with the work.

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TRAMETES CINGULATA BERK. IN CULTURE

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Introduction

Among the Hymenomycetes, causing the decay of woods in our forests, the Polyporaceæ probably cause the greatest amount of damage. In order to control the inroads of these fungi, it is essential that we should have considerable knowledge regarding their biology. Considerable amount of work in this line has already been done in our country, but there are still many of them about which very little is known. *Trametes cingulata* Berk. is one of them. It is a common saprophyte that attacks our structural timber. It grows abundantly on logs and stumps of *Shorea robusta* Gaertn. f., so common in the timber-yards of Calcutta and suburbs. It also attacks some hardwood species and monocotyledons, such as *Grevillea robusta*, *Ficus benghalensis*, *Cocos nucifera* and *Bambusa arundinacea*. Though Bose (1919, 1930, 1937) has reported it from Bengal as a saprophyte on dead branches, prostrate trunks, logs, timber, etc., and also studied certain aspects of its biology, yet very little work has been done so far regarding its responses in culture to variations of environmental factors. The present investigation has therefore been undertaken concerning the influence of a number of contributory factors upon the growth of this organism and the results are presented in the following pages.

Source of Material

The cultures of *Trametes cingulata* Berk. were made from the spores of the sporophores according to the technique followed by Banerjee (1955). A small rectangular portion (10 × 10 mm.) of a freshly collected fruit body was fixed eccentrically with its hymenial surface downwards to the inner side of the upper lid of a sterile Petri dish containing 2·5% sterile cleared agar. Care was taken so that the hymenial surface did not touch the agar while the upper lid of the Petri dish was placed in position. It was then kept at a room temperature of 30° C. within a bell-jar lined inside with moist blotting-paper. Within a couple of hours spore-deposit was obtained on the surface of 2·5% sterile cleared agar in a Petri dish from which several polysporous

cultures were made by transferring loopfuls of spores asceptically to 2·5% malt agar slants. These were kept in the laboratory under identical environmental conditions and within a week a good mycelial growth was obtained in all the tubes. These were kept as stock cultures for study and comparison. All the cultures showed the presence of clamp-connexions and therefore obviously in the second stage of development. All experiments on the cultural characteristics of *Trametes cingulata* have been carried out from cultures obtained from this source.

FUNGUS IN CULTURE

Spore-germination (Text-Fig. 1)

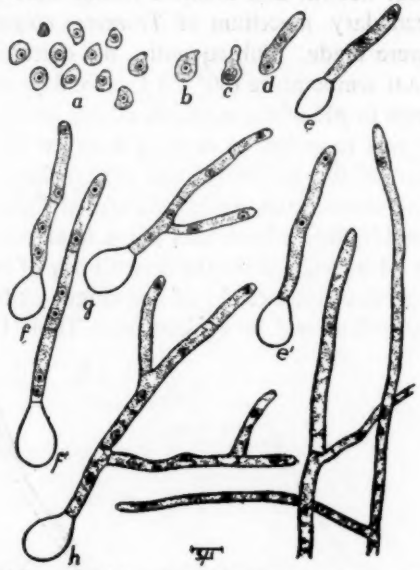
On 2·5% agar medium germination occurred within 10 to 12 hours after planting the spores. The agar plates after spore-shed were placed within a moist chamber in the diffused light of the laboratory and at room temperature (30° C.). This condition seemed quite favourable for the germination of the spores. More than 95% of the spores germinated easily in the above way. Since no difficulty was experienced in getting germination of spores, other methods were not tried for studying the same. Germination started with swelling of the spores to about twice their normal size and subsequent formation of a simple germ-tube (Text-Fig. 1). Usually the germ-tube appeared at the apex of the spore, but sometimes it may also develop from its sides. The germ-tube develops directly into the primary mycelium which is characterised by the absence of clamp-connexions, and by comparatively narrow (2·5 μ wide) hyphæ.

Oxidase test

Trametes cingulata is a ' white rot ' fungus. This was determined after performing the oxidase tests. The oxidase test, as described by Bavendamm (1928), was done by growing the test fungus on malt agar medium containing 0·5% gallic acid or tannic acid in sterile Petri dishes. Fungus causing ' white rot ' forms dark discoloured area around the inoculum and is thus considered as positive reactors, while a fungus causing ' brown rot ' does not form dark discoloured area. It is thus negative in reaction. As decomposition of lignin is, according to Wehmer (1927), an oxidative process, the secretion of oxidase by a ' white rot ' fungus is naturally expected.

The secondary mycelium of *Trametes cingulata* was grown on a 0·5% *malt-agar* medium in sterile Petri dishes (90 mm. in diameter) containing the requisite quantity of tannic acid or gallic acid. In both the cases, dark brown zones appeared due to the presence of oxidase within 36 hours after inoculation. It was observed that the intensity of reaction was somewhat greater

in the tannic acid medium than that in medium containing gallic acid. The diffusion zone in tannic acid medium was *very intense*, dark brown in colour, more or less opaque and forming a wide corona of about 15 mm. in diameter. In the gallic acid medium, on the other hand, this diffusion zone was moderately strong, light to dark brown in colour and was only 8 mm. in diameter as visible from the upper side of the mat. The results of the experiment is summarised in Table I. The descriptive terms indicating the strength of the reactions and also the symbols used are according to Davidson, Campbell and Blaisdell (1938).



TEXT-FIG. 1. Basidiospores (*a*) and their stages of germination (*b-h*), hyphæ of the primary mycelium (*i*, *j*).

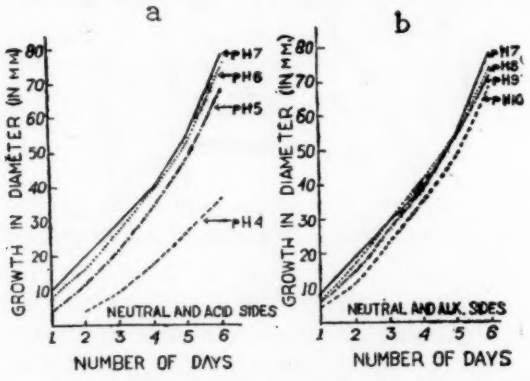
TABLE I

Results of oxidase tests with secondary mycelium of Trametes cingulata

	2·5% *malt-agar* medium with	
	0·5% tannic acid	0·5% gallic acid
Intensity of reaction	Very strong (+++++)	Moderately strong (+++)
Diameter of the diffusion zone	15 mm.	8 mm.

Effects of variation in acidity and alkalinity of the medium

In order to study the effects of variation in the acidity and alkalinity of a medium on cultural characteristics of *Trametes cingulata*, 2·5% *malt-agar* medium was used. The medium with different grades of pH were prepared by following a standard colorimetric method using an universal indicator and N./10 HCl and N./10 NaOH solutions as acid and alkali respectively. Seven different grades of *p*H, three on either side of the neutral (*p*H 7), were thus prepared as follows: *p*H 4, 5, 6, 7, 8, 9 and 10. All the Petri dishes containing sterile acid, neutral and alkaline media were then inoculated at the centre by the secondary mycelium of *Trametes cingulata*. Three replicates of each type were made. Subsequently, the cultures were kept in an incubator at a constant temperature (30° C.) for six days and a month thereafter to note the change in *p*H of the medium, during which time the humidity inside the incubator was recorded as ranging between 50% and 65%. The external wooden door of the incubator was always kept open so that the cultures received approximately six hours of diffused light through the glass door every day. Except in the medium with *p*H 4, in all other cases the fungus began to grow within 24 hours, and on the seventh day of inoculation the rate of growth and the growth characteristics of the fungus under aforesaid conditions became well marked, as will be evident from Table II and Text-Fig. 2.



TEXT-FIG. 2. Graphs showing effects of acidity (*a*) and alkalinity (*b*) on the rate of growth of *Trametes cingulata*.

From Tables I and II it becomes evident that the maximum growth in diameter of the superficial mat takes place at *p*H 7; it gradually declines both towards the acid and the alkaline sides. A striking feature of this fungus is its tolerance of both acid and alkaline conditions, growth being more pronounced towards the alkaline side (*p*H 8, 9, 10) than that towards the acid (*p*H 6, 5, 4).

TABLE II

Cultural data on the seventh day of inoculation due to acidity and alkalinity of the medium showing growth characteristics of secondary mycelium of Trametes cingulata

*p*H values	Texture	Colour	Growth in dia. (mm.)
4	Raised, subfelty; not zonal; advancing zone not clearly differentiated	Slightly orange	38
5	Raised, subfelty; not zonal; with radiating fusiform streaks; advancing zone adpressed, small, about 1–2 mm.	White	70
6	Raised, subfelty; faintly zoned; with radiating fusiform streaks; advancing zone adpressed, about 2–4 mm.	White	76
7	Raised, subfelty to felty; prominently zoned; with radiating streaks; advancing zone adpressed, about 5 mm.	White	80
8	Raised, felty; not zoned; with radiating fusiform streaks; advancing zone adpressed, about 4–5 mm.	White	76
9	Raised, subfelty to felty; faintly zoned; with radiating streaks; advancing zone adpressed, about 4–5 mm.	White	74
10	Do.	White	70

Since no buffer had been used, it was not possible to maintain the media at their respective *p*H values during the progress of the experiment. The change in *p*H value in each of the seven different grades were, however, noted after a lapse of 30 days as indicated in Table III.

Cultural characteristics on different media

In order to study the effects of different media on cultural characteristics of *Trametes cingulata* under a set of constant conditions, the following media were selected: (i) *potato-dextrose-agar* (according to Fritz, 1923), (ii) *malt-agar* (malt extract 2·5%, agar 2·5%, distilled water 1,000 c.c.), (iii) *Czapeck's synthetic-agar* as modified by Fritz (1923), and (iv) *wood-decoction-agar*. The *wood-decoction-agar* was prepared according to the

TABLE III

Changes in pH values of the media before and after (30 days) the experiment

*p*H value of the medium before the experiment	*p*H value of the medium after the experiment
4	5·1
5	5·7
6	5·9
7	6·5
8	6·9
9	7·4
10	7·8

following composition. As the fungus was found to be growing luxuriantly on logs of *Shorea robusta*, its sawdust from healthy wood was taken for the preparation of the medium. Fifty gm. of oven-dried sawdust and 2·5% agar per litre of distilled water were used and the medium was prepared in the usual way. All the media were then standardised to *p*H 7 by following the method already described before. These were sterilized in an autoclave at 15 lb. pressure for 20 minutes. After sterilization *p*H values in all the media were checked and found to be very near the neutral point and therefore taken as negligible. The media were timely plated in sterilized Petri dishes (90 mm. dia.) and each inoculated at the centre by the secondary mycelium of the fungus. Three replicates for each medium were made and the Petri dishes were kept in an incubator at a constant temperature of 30° C. The humidity inside the incubator ranged between 50% and 65%. A set of cultures were kept in complete darkness and another in diffused light of the laboratory. The latter condition was achieved by keeping the wooden door of the incubator open during the experimental period so that the cultures received diffused light for about six hours each day through the glass door. The observations were continued for a period of seven days until the mycelia nearly reached the edge of the Petri dishes. The cultures were kept under the conditions for a further period of 21 days to note subsequent changes in their cultural characteristics. The observations are summarized as follows;—

(*i*) *Habit of growth.*—(*a*) On *potato-dextrose-agar* the mycelium starts growing rapidly within 24 hours in diffused light. By the second day, the mycelium forms a distinct adpressed and sodden growth, about 3–4 mm. wide, along the inoculum. Over the inoculum, on the other hand, the growth is mealy to farinose. On the 4th day the mycelium becomes somewhat raised, and condenses gradually and differentiates into a subfelty zone towards the inoculum, while the zone of advance remains adpressed as before. Soon alternate narrow and wide concentric zones of subfelty and thin mycelia make their appearance. Meanwhile due to continued condensation of the aerial mat the subfelty zones, on the 7th day of inoculation, become felty. In darkness, mycelial growth starts similarly within 24 hours and on the 2nd day an adpressed advancing mycelium of about 5–6 mm. wide is found around the inoculum and this, as growth continues, gradually condenses into a raised subfelty to felty mycelium. On the 4th day, the uniform subfelty texture of the mat is interrupted and radiating streaks of felty mycelia make their appearance. On the 7th day, these streaks become more prominent and somewhat fusiform in shape. In no case zonation of the mat is observed.

(*b*) On *malt-agar* also mycelial growth becomes evident within 24 hours in both diffused light and darkness. In both cases, over the inocula the mycelium is subfelty and around the medium, on the 2nd day, a sodden adpressed mycelium is formed. Gradually, the latter becomes raised and uniformly subfelty but without any distinct zone of advances. On the 7th day, in diffused light, the mat is much raised and forms a distinct zone at the periphery. In darkness, on the other hand, it shows more or less similar growth and texture throughout, but on the 7th day prominent wedge-shaped, radiating, felty streaks appear and are separated from one another by hyaline area.

(*c*) On *Czapeck's synthetic-agar*, under identical conditions, growth is relatively slow and thin. Over the inoculum, the mycelium is subfelty from the beginning. Both in diffused light and in darkness, on and from the 2nd day, thin semitransparent and somewhat adpressed mycelia gradually grow out and a few indistinct zonations are formed. On the 4th day, radiating hyaline streaks with somewhat condensed white mycelium in between them are formed in diffused light. In the darkness, on the other hand, uniformly subfelty areas are found to be wedged between the hyaline arms. Condensation is poor in both the cases. The zone of advance is also present, which is hyaline or semi-transparent. In both cases, a few zonations are formed. The radiating streaks appear as usual and gradually become prominent, but in darkness, they give the mat a star-like appearance on the 7th day of inoculation.

(*d*) On *wood-decoction-agar*, growth is very slow under similar conditions. From the 2nd day of inoculation, mycelial growth is noticed over the medium, but from the beginning it is raised, subfelty and uniform in texture throughout and remain in that condition even up to 5 days. No zonation is visible either in diffused light or in darkness. Further condensation of the mat is not observed. On the 7th day, under both diffused light and darkness, the peripheral portion of the mat shows faint striations. An interesting aspect of growth on this medium is the formation of oxidase rings, in both cases, from the very first day of inoculation.

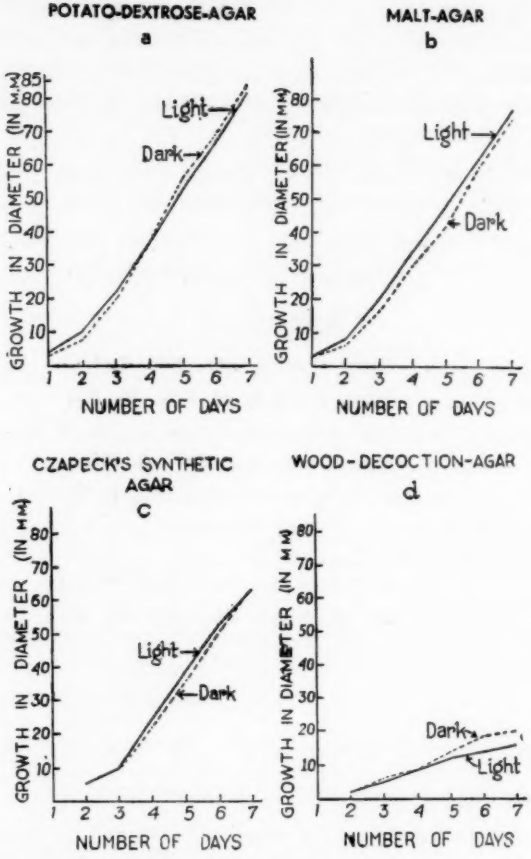
On further study of all the cultures for 21 days thereafter the following observations were made. The superficial mycelium is more condensed in *potato-dextrose-agar* and *malt-agar* than that in the other two media, and shows a tendency towards a subfelty to felty growth. Zonations are markedly prominent in *potato-dextrose-agar*, becoming less prominent in *malt-agar* and *Czapeck's synthetic-agar*, while those in *wood-decoction-agar* are almost indistinct. Radiating streaks are always present to a greater or lesser degree in all the cases.

(*ii*) *Colour.*—Except in the *wood-decoction-agar*, in all other cases the mycelium is hyaline at first and as condensation sets in it gradually becomes whiter. In the *wood-decoction-agar*, on the other hand, the mycelium is white over the inoculum, but over the medium it is slightly with a brownish tinge from the beginning, which is possibly due to oxidase reactions. Even in cultures ,30-days-old, no pigmentation is ever noticed and the mycelium remains uniformly white throughout.

(*iii*) *Rate of growth.*—Maximum daily rate of growth of the superficial mycelium is obtained on *potato-dextrose-agar*, being followed by *malt-agar*, *Czapeck's synthetic-agar* and *wood-decoction-agar* in succession. This will be evident from Text-Fig. 3.

From the aforesaid observations, it would appear that *potato-dextrose-agar* is the best medium for the cultivation of *Trametes cingulata*, both as regards the texture of the mat and the rate of growth. Light also seems to exert some influence on growth characteristics of the fungus under identical conditions. In absence of light, the growth characteristics are more uniform in all the media, radiating streaks appear in all cases and zonations are usually absent. In diffused light, on the other hand, zonations invariably appear in all the cultures and the radiating streaks are less prominent. These zonations of the mat are formed possibly due to alternate effects of diffused light and darkness to which the cultures were subjected. In darkness, on the

other hand, the conditions are more uniform and as such no concentric bands of superficial mycelium are formed.



TEXT-FIG. 3. Graphs showing effects of different media (*a–d*) on the rate of growth of *Trametes cingulata* in light and darkness.

SUMMARY

1. Growth characteristics of *Trametes cingulata* Berk. have been studied in culture under different environmental conditions.

2. Basidiospores from freshly taken fructifications have been found to germinate very quickly on agar medium under ordinary conditions of diffused light and temperature (30° C.) of the laboratory. Basidiospores always germinate by means of a single germ-tube.

3. Being a ' white rot ' fungus, it gives positive reaction to Bavendamm's oxidase test.

4. The fungus has been grown on 2·5% *malt-agar* medium with seven different grades of pH, three on either side of the neutral. The best growth has been found in the medium having neutral *p*H value. An interesting feature is the wide range of tolerance of the fungus to both acidic and alkaline conditions to a certain extent.

5. Cultural characteristics of the fungus have been studied on four different media under a set of constant conditions. Best growth has been obtained on *potato-dextrose-agar*, lesser in *malt-agar*, then *Czapeck's synthetic-agar* and *wood-decoction-agar* following in succession.

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THE MEASUREMENT OF CARBON DIOXIDE PRODUCED BY AIR-BREATHING FISHES AND EVIDENCE OF THE RESPIRATORY FUNCTION OF THE ACCESSORY RESPIRATORY ORGANS

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Introduction

In earlier studies little direct evidence has been given to establish the respiratory function of the accessory respiratory organs. In most cases, the significance of the air-breathing function is based on anatomical and biological rather than physiological evidence. An extensive literature exists on the evolution of respiratory organs, their anatomy, functional modifications and adaptive significance. A great deal of these works is devoted mainly to the bionomics of various air-breathing forms (Das, 1933 and 1934; Carter and Beadle, 1931).

To establish, however, the air-breathing function of the accessory respiratory organs, a variety of indirect evidences has been given (Das, 1934). These can be summarized as follows:

1. "Asphyxiation of the fish by preventing access to the air."
2. "Recovery after drowning by exposure to aerated water."
3. "Effect of injury to special air-breathing organs."
4. "Survival out of water."

All these evidences are undoubtedly valuable and, in many cases, provide proof of the air-breathing function. Nevertheless, a fuller understanding can only be gained by measuring the respiratory activity of the air-breathers. Data on the respiratory rate are not very numerous and it is seldom that reliable methods have been worked out (Krogh, 1941; Carter, 1958). Krogh (1904) produced some experimental evidence on the aerial respiration of eel by measuring the oxygen uptake and carbon dioxide excretion. He thus concluded that in this fish, the skin acted as an organ of breathing air.

In the present investigation an attempt has been made to obtain quantitative data on the amount of carbon dioxide produced by some air-breathing fishes outside water. The scope of the present work is only limited to the technical details and the working of the apparatus. The results neither determine the total amount of oxygen consumed nor the extent of respiration carried out by the general body surface. But the fact that a definite and measureable quantity of carbon dioxide is produced by these fishes shows that the air-breathing organs are capable of performing an effective respiration outside water.

METHODS AND DESCRIPTION OF THE APPARATUS

A general plan of the apparatus is shown in Fig. 1. Fishes were kept in an empty air-tight experimental flask and the air contained in the flask was removed by means of a vacuum pump. The extent of vacuum created

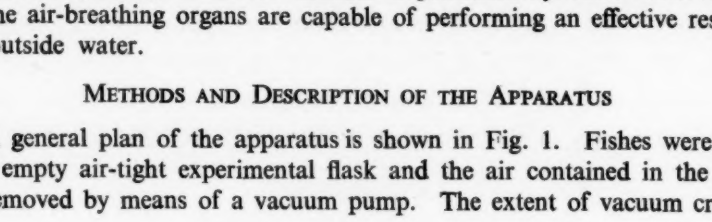

FIG. 1. A general plan of the apparatus designed to measure the amount of CO_2 produced by the air-breathing fishes outside water. The arrows indicate the directions of evacuation of the experimental flask and its filling with CO_2-free air.

was measured with the help of an attached mercury manometer. Carbon dioxide-free air was then passed in the flask by means of an aerator. The removal of carbon dioxide from air was done by passing it through two bottles containing strong KOH solution (N strength) and a U tube containing solid KOH.

To ensure that the experimental flask contained nothing but the carbon dioxide-free air, the operations of filling the flask and its evacuation were repeated several times. The pressure of carbon dioxide-free air in the flask was measured by means of an attached water manometer (Fig. 1). All connections of the apparatus were made by pressure rubber tubings and each joint was wired tightly. Thus the entire apparatus became air-tight.

Fishes were allowed to respire in the flask containing carbon dioxide-free air for a given length of time (the pressure of CO_2-free air in the flask

was kept slightly above the atomosphere). The total amount of carbon dioxide produced by the fishes during respiration was finally evacuated through U tubes containing $CaCl_2$ and KOH. The extent of evacuation of the flask was again measured by the same mercury manometer. The weight of the KOH tube having been carefully measured previously, any increase in its weight gave an index of the amount of carbon dioxide produced. The other tube containing $CaCl_2$ was used only to absorb any moisture produced during experiments. It thus made the air contained in the flask free from moisture before entering the KOH tube.

All fishes were carefully weighed before they were introduced into the experimental flask, and roughly 150–200 gm. of live weight was taken in each experiment. Special care was exercised to see that all fishes of one species were approximately of the same size. Readings of the total amount of CO_2 produced were taken after a lapse of six, twelve, eighteen and twenty-four hours. For each reading, either freshly collected specimens were taken or those which had fully recovered from the effects of earlier experiments. In summer months, cases of mortality appearing at the end of each experiment, particularly when the fishes were subjected to experimental conditions over prolonged periods, were quite frequent. To overcome this difficulty, the experiments were discontinued during the hot weather. In winter, on the other hand, no mortality was ever encountered and for this reason all the data included in the present investigation refer to four months only—November to February.

Behaviour of Fishes During Experiments

The removal of air from the flask and its final evacuation through the $CaCl_2$ and KOH tubes after the respiration had taken place involved a reduction in pressure of about one atmosphere. When the pressure reached nearly half an atmosphere, the fishes showed signs of excitement. On further reduction of pressure, symptoms of shock became more apparent. Some began to lie on their sides whereas others continued to wriggle and jump for a time and then quietened down. Soon after the evacuation was over and the supply of CO_2-free air was maintained, recovery began, and about 20 minutes later things were back to normal. The recovery of fishes in the flask containing CO_2-free air was remarkable. Fishes which appeared to be in a state of shock soon became active and lively.

After the experiment, the fishes were returned to well-aerated water. On transference to water, some became buoyant whereas others lost their balance completely. A few which remained still active, went to

the surface frequently to gulp air and finally swam to the bottom. The buoyant condition was more commonly seen when the fishes were kept in the experimental condition for prolonged periods. Such fishes remained vertically suspended in water for about an hour or so. Their breathing movement became very slow and feeble. Gradually they regained their sense of balance and started swimming. The next day they seemed quite normal.

RESULTS

The respiratory process of the following three species was investigated:

1. *Ophicephalus punctatus* Bloch.
2. *Clarias batrachus* (L.)
3. *Heteropnuestes fossilis* (Bloch.)

Ophicephalus punctatus

As compared to the other two species, this fish was most susceptible to its survival in the flask during experiments. Changes of pressure also seemed to affect the fishes rather seriously. In the hot weather, many fishes died during the experiment and it is seldom that a 24-hour reading reached completion without an accident in this season. However, as noted above, no mortality ever occurred during the winter months.

The weight of the CO_2 produced per unit body weight is given in Table I and the mean values have been plotted in Fig. 2. As can be seen from Table I

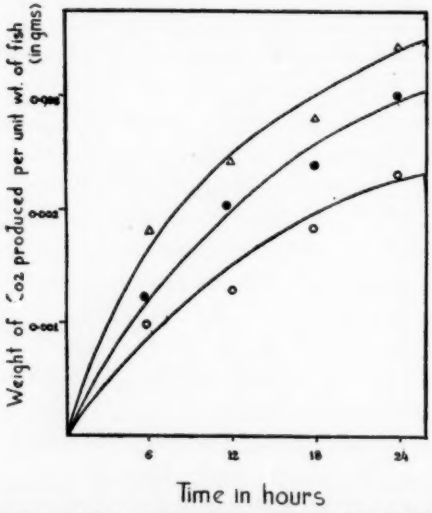

FIG. 2. The mean values of CO_2 produced per unit weight of fish at successive intervals O—*Ophicephalus punctatus.* ●—*Clarias batrachus.* △—*Heteropnuestes fossilis.*

and Fig. 2 relatively less quantity of CO_2 is produced by this species. All members of this genus possess pharyngeal diverticula as organs of breathing air. This type of respiration is known as bucco-pharyngeal or mouth-breathing (Das, 1940; Carter, 1951). This is the simplest and the most primitive type of air-breathing known in fishes. In structure, the respiratory organ consists merely of an epithelium of the mouth cavity and pharynx, richly supplied with blood vessels.

TABLE I

Total weight of CO_2 produced per unit weight of Ophicephalus punctatus *at successive intervals*

Time in hours	6	12	18	24
Weight of CO_2 produced in grams ..	0·000972	0·001341	0·001841	0·002250
	0·001004	0·001316	0·002005	0·002305
	0·001041	0·001368	0·001734	0·002288
Mean ..	0·001005	0·0013418	0·001860	0·002281

Clarias batrachus

During experiment no serious disturbance was noticed in the behaviour of this species. Some of the individuals, however, showed signs of discomfort by the low pressure changes in the flask and frequently fell over on their sides. After the experiments, on return to water, they remained suspended for a long time before resuming their normal behaviour.

Table II and Fig. 2 give the weight of CO_2 produced per unit body weight and their mean values respectively. It is evident from the table and the figure that the rate of CO_2 production in this species was higher than that of *O. punctatus*. These fishes have evolved a complicated set of accessory respiratory organs in the form of paired pharyngeal lungs with " Coral-like rasettes or air trees ".

Heteropnuestes fossilis

Apart from a little excitement, while the flask was being evacuated, no other change in the behaviour of this species was noticed during experiment. After the experiment, on transference to water, they only showed signs of fatigue and in many cases symptoms of disturbed sense of balance also appeared. Undoubtedly, this species seems best suited for life out of water as

compared to the other two noted above. The production of CO_2 as indicated in Table III and Fig. 2 is higher than those of *O. punctatus* and *C. batrachus*. The organs of breathing air in this species are highly evolved, massive paired sac-like pharyngeal lungs extending backwards up to the middle of the tail regions.

TABLE II

Total weight of CO_2 produced per unit weight of Clarias batrachus *at successive intervals*

Time in hours	6	12	18	24
Weight of CO_2 produced in grams ..	0·001198	0·001968	0·002369	0·003084
	0·001244	0·002080	0·002418	0·002757
	0·001201	0·002068	0·002468	0·003145
Mean ..	0·001214	0·002038	0·002418	0·002995

TABLE III

Total weight of CO_2 produced per unit weight of Heteropneustes fossilis *at successive intervals*

Time in hours	6	12	18	24
Weight of CO_2 produced in grams ..	0·001947	0·002546	0·002834	0·003246
	0·001675	0·002568	0·002781	0·003446
	0·001880	0·002277	0·002680	0·003334
Mean ..	0·001834	0·002463	0·002765	0·003342

CONCLUSIONS

The differences in the CO_2 produced by the three species noted above fall in close agreement with the structural complexities and respiratory efficiencies of their accessory respiratory organs. Species possessing a simple type of organ, *e.g.*, *O. punctatus* give out less CO_2 and their survival out of water is limited to a shorter duration. Complex and highly developed air-

breathing organs such as in *C. batrachus* and *H. fossilis* produce more CO_2 and these species survive out of water over a prolonged period.

The rate of production of CO_2 in all the three species decreases in relation to time (Fig. 2). The first six hours, however, are enough to produce a significant change. At longer intervals its production continues at a slower rate and after 18 hours there is only a slight increase.

Summary

By taking the amount of CO_2 produced as an index of respiratory activity outside water, the air-breathing function of the following species has been demonstrated:

1. *Ophicephalus punctatus.*
2. *Clarias batrachus.*
3. *Heteropnuestes fossilis.*

O. punctatus, which possesses paired pharyngeal diverticula as organs of breathing air, produces the least quantity of CO_2. In *C. batrachus* and *H. fossilis* the production of CO_2 is relatively higher. Both these species possess opercular lungs. The differences in the CO_2 produced between the three species fall in close agreement with the structural complexities of their accessory respiratory organs.

The rate of production of CO_2 in all the three species decreases in relation to time. After 24 hours most of the fishes become moribund but soon recover when kept in aerated water.

Acknowledgements

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STUDIES ON FOLIAR SCLEREIDS IN DICOTYLEDONS

VII. Origin and Development of Sclerenchymatous Idioblasts in the Leaf of *Manilkara hexandra* (Roxb.) Dubard.

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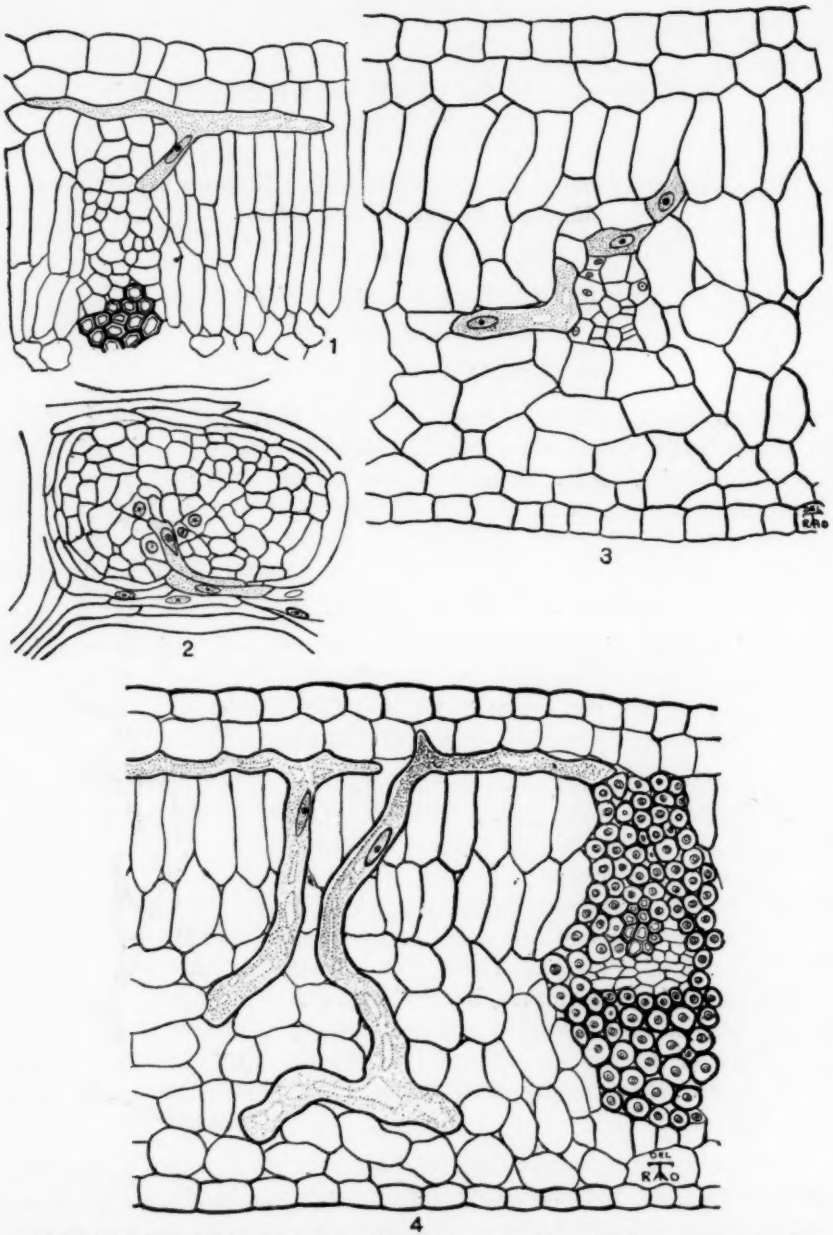
Sclerenchymatous fibres in close association with the veins and veinlets forming a regular strand are widely distributed in various families of Angiosperms (Solereder, 1908). Sometimes they exhibit bizarre cell forms much like idioblastic sclereids at the tips of veinlets in a prevailingly terminal position (Rao, 1951, Fig. 10). This interesting topography of sclerenchymatous idioblasts prompted me to investigate their ontogeny and development, an account of which is presented in this paper.

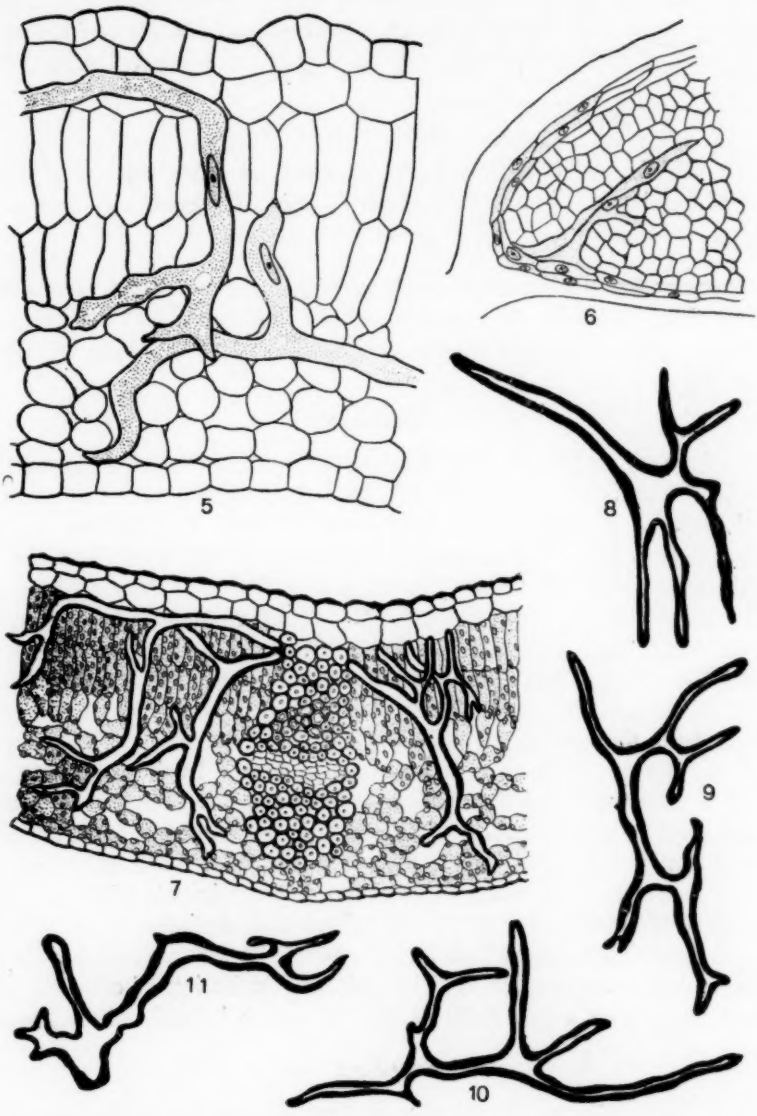
Leaf material of *Manilkara hexandra* was collected in different regions of Poona, Coonor, Bangalore and Ahmedabad. The fixed materials were microtomed by the customary methods. The technique of staining and clearing the leaves is outlined in the previous paper (Rao, 1957).

Sclerenchymatous fibres in the mesophyll are widely distributed in the family Sapotaceæ. Holle (1892) describes them in *Chrysophyllum glabrum* Jacq., in all species of the section *Caloneuron*, in certain species of the section *Chrysalidops*, in the genus *Micropholis* and in certain species of *Payena*, *Bumelia*, *Mimusops*, *Oxythece*, *Pachystela*, *Achras*, *Passaveria*, *Labourdonnaisia*, *Sersalisia*, *Lucuma*, *Amorphospermum*, *Labramia*, *Elæoluma* and *Cymnoluma*. These sclerenchymatous fibres generally have a thick wall and narrow lumen, but moderately thick fibres with wide lumina are met with in *Chrysophyllum glabrum*; spirally thickened fibres occur in the species of *Micropholis* belonging to the section *Caloneuron*. Engler (1904) records sclerenchymatous fibres in the mesophyll in *Synsepalum delciforme* Daniell.

Distribution of Sclerenchymatous Idioblasts in the Lamina.—Cleared leaf preparations show that sclerenchymatous strands are found in close contact with the major and minor veins; oftentimes they deviate from their path to



FIGS. 1–4. Trans and paradermal sections of young and immature leaves. Fig. 1. Growing sclerenchymatous idioblasts; note inter-epidermal branching and slightly sclerosed wall with a broad lumen, ×450. Figs. 2–4. Stages in formation of sclerenchymatous idioblasts, ×450 each.



FIGS. 5–11. Fig. 5. Transection of immature leaf showing growing sclerenchymatous idioblasts, ×450. Fig. 6. Paradermal section of immature leaf, ×450. Fig. 7. Transection of a mature leaf, showing disposition of sclerenchymatous idioblasts, ×225. Figs. 8–11. Polymorphic sclerenchymatous idioblasts, ×300.

traverse into the adjacent mesophyll cell. Such features have been noticed in *Ochna squarrosa* L. (Ochnaceæ), *Uvaria macrophylla* Roxb. and *Cynometra ramiflora* L. (Cæsalpinieæ) (Rao, 1953) and *Quiina acutangula* Ducke (Foster, 1950).

The study of macerated material revealed the existence of irregularly shaped cell forms (Figs. 8–11). In transections they are conspicuously disposed inside the lamina; sometimes touching the epidermal layers, and running intra-epidermally with or without branches (Fig. 7).

Irregular forking is a common feature in all the cell forms. Irrespective of their form variation, they have a uniform thick wall with varying width of lumen. The cell-wall is closely striated and exhibits straight or oblique pit canals.

ONTOGENY

In the early stages, when the lamina does not show tissue differentiation, the cells are more or less closely arranged with minute airspaces. Careful examination of a number of young stages revealed the presence of procambial strands in an active stage of differentiation into xylem and phloem. At this stage some cells adjacent to the procambial cells show a strong tendency to deviate into the adjoining cells. They possess conspicuous pear-shaped nuclei and dense protoplasm. These are the sclerenchymatous initials (Figs. 1–6). Soon they become tubiform and exhibit individualistic growth. The growth is intercellular and forking is very often seen at such places where the mesophyll cells are devoid of air-clefts. The nucleus occupies different positions in the growing idioblast. In the next stage they branch out extensively in the mesophyll and possess a smooth wall without any spicules. An examination of paradermal and transections at this stage revealed that the growing initials are often forked at the terminal or subterminal positions of veinlets, whereas the growing initials which are close to the differentiating major veins, apart from their deviation into the mesophyll, do not show bizarre cell forms. With the stoppage of growth the initials undergo sclerosis followed by uniform thickening of cell-wall. The nucleus persists till a late stage of lignification and finally disintegrates.

SUMMARY

The mature leaves of *Manilkara hexandra* exhibit sclerenchymatous idioblasts in close proximity to major veins and veinlets. It is found that the sclerenchymatous initials are transformed spongy cells near the differentiating procambial strands.

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SOURCE OF ADRENALINE AND NORADRENALINE IN THE FROG'S HEART ON STIMULATION OF THE SYMPATHETIC NERVES

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Stimulation of sympathetic nerves to the frog's heart liberates an adrenaline-like substance (Loewi, 1921, 1936, 1937), which is likely to be noradrenaline (Euler, 1956). It is presumed that this is liberated by the sympathetic nerve-endings, though it might be liberated from some near-by stores, such as chromaffin cells (Burn and Rand, 1960).

There is evidence that the neurohormone acetylcholine might be liberated from the muscle cells on nervous stimulation. When the vagus nerves to the frog's and dog's stomach muscle are maximally stimulated and the nerve-endings fatigued, direct, electrical stimulation of the muscle releases enormous amounts of acetylcholine (Singh, Sharma and Bhatnagar, 1959). Similar stimulation of the frog's heart also releases acetylcholine, after fatigue of the vagus nerve-endings (Singh, Singh and Bhatnagar, 1960 *a*). In the frog's heart, the lower half of the ventricle, which is free from ganglion cells, releases more acetylcholine than the upper half of the heart consisting of the upper half of the ventricle, auricles and the sinus venosus and also containing ganglion cells; the acetylcholine content of the ganglion-free portion of the frog's heart is also higher than that of the portion which contains ganglion cells (Singh, Singh and Bhatnagar, 1960 *b*). This definitely shows that acetylcholine is contained in the muscle cells and liberated therefrom.

The above experiments do not exclude the release of acetylcholine from the vagal nerve-endings, though certain observations cast doubt on such a release. The frog's heart may stop on stimulation of the vagus nerve without any detectable release of acetylcholine (Singh, Singh and Bhatnagar, 1960 *a*). If frog's heart is immersed in Ringer's solution containing various concentrations of acetylcholine up to 1 in 10,000, it may continue to beat and yet on stimulation of the vagus nerve, complete inhibition is produced (Singh, Sehra and Singh, 1945). The same has been found on mammalian heart

(Öberink and Essex, 1953), vagal stimulation could stop the heart even when it was beating under the influence of high concentrations of acetylcholine (up to 1 in 100). This can be interpreted in two ways: either the nerve did not liberate acetylcholine, or liberated it within an area into which the acetylcholine, from outside had difficulty in penetrating. In the frog's heart this is unlikely, as the tissue is thin and transparent; at least the experiment excludes the extracellular release of acetylcholine by the vagal nerve-endings.

Both the mammalian and frog's heart contains noradrenaline and adrenaline. Goodall (1951) found that in the sheep's heart, there was a mean amount of 0·79 μg./g. noradrenaline and 0·15 μg./g. adrenaline. Frog's heart (*Rana temporaria*) contains 0·10 to 0·34 μg./g. noradrenaline and 0·76 to 1·00 μg./g. adrenaline (Östlund, 1954).

On stimulation of the vagus nerve in the frog's heart, the acetylcholine appears to come from the muscles. In the present research an attempt has been made to determine the source of adrenaline and noradrenaline in the frog's heart on stimulation of the sympathetic nerves.

EXPERIMENTAL

These experiments were performed on the heart of the frog, *Rana tigrina*. The heart was dissected out with both the vago-sympathetic nerves attached. It was washed free of blood by repeated washing with Ringer's solution. It was then placed in a small petri-dish containing 12 c.c. of Ringer's solution for one hour; it usually beats spontaneously. This was done for one hour to note any liberation of adrenaline and noradrenaline by the spontaneously beating heart; the heart beats for about 2 to 3 hours. The Ringer's solution was then replaced afresh and both the sympathetic nerves were placed on a pair of electrodes and stimulated maximally with induction shocks for one hour. At the end of period of stimulation, the Ringer's solution was collected and tested for adrenaline and noradrenaline.

The nerves were then cut out and the heart placed in a small muscle chamber containing 12 c.c. of Ringer's solution and stimulated with induction shocks at the rate of 16 times per minute. The Ringer's solution was then tested for adrenaline and noradrenaline. To minimise the oxidation of adrenaline and noradrenaline, the heart was aerated with air instead of oxygen and the Ringer's solution was unbuffered, the bicarbonate being omitted.

To estimate adrenaline and noradrenaline, extracts were prepared as described by Vogt (1952) for blood. Ringer's solution for analysis was acidified with concentrated hydrochloric acid by adding 0·1 c.c. of the acid to 100 c.c. of the solution. This solution was concentrated *in vacuo* to 2 c.c.

at bath temperature of 45 to 50° C. To the concentrated solution, 14 c.c. of acidified ethanol (0·1 c.c. of concentrated hydrochloric in 100 c.c. of ethanol) was added. The mixture was chilled in refrigerator for one hour. It was then centrifuged for 5 minutes (3,000 revolutions per minute). The supernatant was transferred to 50 c.c. round-bottomed flask. The precipitate in the centrifuge tube was washed with more ethanol, chilled, centrifuged and the supernatant added to the previous one. The combined supernatant was evaporated just to dryness *under vacuo* at bath temperature of 45 to 50° C.

The residue in the flask was detached with sodium chloride saturated ethanol (total volume, 6 to 7 c.c.), and transferred to a 15 c.c. centrifuge tube. It was chilled for 10 to 15 minutes and then centrifuged. The supernatant was taken in a 50 c.c. round-bottomed flask. The precipitate in the centrifuge tube was washed with 1 to 2 c.c. of sodium chloride saturated ethanol, chilled, centrifuged and supernatant added to the previous. The combined supernatant was evaporated just to dryness *in vacuo* as before. The residue was detached with the help of four portions of 0·5 c.c. of ethanol-acetone mixture (1 : 1). The suspension was cooled and centrifuged. The supernatant was applied to the filter-paper for chromatography. The residue in the centrifuge tube was washed with 0·25 c.c. of acetone-ethanol mixture, cooled, centrifuged and the supernatant applied to paper.

The extract from the heart was prepared according to the procedure described by Goodall and Long (1950) and originally described by Euler (1948). The frog was pithed and the heart removed as soon as possible. It was cut into two parts longitudinally and washed with distilled water to remove blood. It was weighed immediately and placed in a glass mortar containing 5 per cent. trichloracetic acid (two volumes of acid to one volume of heart). The heart was ground in the mortar with sand. This was filtered and to the filtrate, 20 per cent. aluminium sulphate was added, the amount of aluminium sulphate being 2 per cent. of the filtrate volume. Sodium hydroxide (0·5 per cent. solution) was added slowly to the filtrate to form aluminium hydroxide precipitate. Adrenaline and noradrenaline are adsorbed on the precipitate. The adsorbability of adrenaline and noradrenaline by aluminium hydroxide in mildly alkaline solution depends upon the integrity of the catachol nucleus since adrenochrome and noradrenochrome are not similarly adsorbed (Raab, 1943). The precipitate formed is filtered off, washed with distilled water and dissolved with the aid of 2 N sulphuric acid; generally 2 to 3 c.c. is sufficient. The pH of the extract was made to 3·5 by slowly adding 0·5 N sodium hydroxide and stirring during addition.

To the extract was added acetone-ethanol mixture (1 : 1) in the ratio of one volume of extract and four volumes of acetone-ethanol mixture. The whole was chilled in refrigerator for 10 hours to precipitate the salts. This was then filtered and the filtrate evaporated *in vacuo* at bath temperature 40 to 45° C. just to dryness. The residue was detached with two portions of 0·5 c.c. acetone-ethanol mixture, cooled and centrifuged. The supernatant was applied to paper for chromatography. The precipitate in the centrifuge tube was washed with 0·25 c.c. of acetone-ethanol mixture, centrifuged and supernatant applied to paper.

For separation of adrenaline and noradrenaline by paper chromatography the method of Crawford and Outschoorn (1951) was followed. In these experiments, the chromatogram was allowed to develop at room temperature which was between 25 and 33° C. When the temperature was high, as in summer, the ascent of phenol was greater and separation was complete in less time than in winter (usually within 16 to 18 hours), as also found by Vogt (1952).

The method of preparation of eluates was the same as described by Crawford and Outschoorn (1951) with few differences: (1) Eluates from paper strips were evaporated *in vacuo* at 40 to 45° C. external bath temperature instead of evaporating at 55 to 60° C. Care was taken to avoid continued heating of dried-out residue on the side of the flask because in that case there was loss in adrenaline and noradrenaline content of the eluates, as also reported by Goldenberg, Sertin, Edwards and Rapport (1954). (2) The residue in the flask was taken up in 0·5 c.c. of ascorbic acid solution (50 μg./c.c.) as the quantity of adrenaline and noradrenaline was very small.

The assays were done on rat's blood pressure. The method described by Crawford and Outschoorn (1951) was followed with few differences. Albino rats weighing between 200 and 300 g. were used. They were anæsthetised with urathane 175 mg./100 g. body weight (25 per cent. solution) given subcutaneously. After complete anæsthesia, the rat was tied to the operation table in supine position. The trachea was cannulated with glass tracheal cannula of external diameter 2·5 mm. One carotid artery dissected ready for cannulation, femoral vein was exposed and was cannulated with special polythene tubing attached to a syringe needle which in turn was joined by a rubber tubing to 10 c.c. microburette containing normal saline. The cannula was fixed firmly to the thigh.

Heparin (200 units/100 g. body weight) was injected through the venous cannula and was washed with saline. Carotid artery was then cannulated

with a polythene tubing attached to a syringe needle which was connected by a rubber tubing to a special Condon's mercury manometer.

Atropine sulphate 1 mg. (1 : 1,000) was injected subcutaneously before cannulation (Beraldo, 1958). In some experiments, atropine 100 μg./100 g. was given intravenously slowly over 2 minutes and the animal left for 10 minutes to recover (Outschoorn, 1952). Hexamethonium tartarate, 10 mg./kg. was given intravenously slowly over 2 to 3 minutes (Outschoorn, 1952). The blood pressure fell and remained constant at low level. The rat was then immediately used for assays.

Test samples containing adrenaline were assayed against standard adrenaline and those containing noradrenaline against standard noradrenaline. Test and standard solutions were injected into the venous cannula by means of 1 c.c. tuberculin syringe. The volume injected at any time was kept constant at 0·4 c.c. In bioassay, matching was done by " bracketing " the unknown sample with two doses of standard differing by a factor of 2.

Results

The adrenaline content of 3 frog's heart (*Rana tigrina*) was 0·178, 0·134, 0·117 μg./g. of tissue respectively; the noradrenaline content was 0·178, 0·245, 0·218 μg./g. These results are different from those on *Rana temporaria* which contains more adrenaline than noradrenaline (Östlund, 1954); the heart from *Rana tigrina* also contains more acetylcholine than that from *Rana temporaria* (Singh, Singh and Bhatnagar, 1960 *b*).

The spontaneously beating heart.—The spontaneously beating frog's heart continuously releases adrenaline and noradrenaline in almost equal quantities (Table I). Acetylcholine is not released (Singh, Singh and Bhatnagar, 1960 *a*). The frog's heart therefore appears to require the release of adrenaline and noradrenaline for its rhythmic activity. The isolated perfused rabbit's heart releases 4 times more adrenaline than noradrenaline (Day, 1956). There is, thus, some difference between the frog's and mammalian hearts in this respect.

Effect of sympathetic stimulation.—Stimulation of sympathetic nerves releases both adrenaline and noradrenaline (Table I). The amount of noradrenaline released is greater than that of adrenaline. The period of maximal stimulation, that is, one hour, is sufficient to fatigue the nerves, as shown by the decline in the output of adrenaline and noradrenaline with continued stimulation.

Effect of direct stimulation.—Direct electrical stimulation immediately after nervous stimulation causes release of adrenaline and noradrenaline in

amounts greater than that caused by nervous stimulation (Table I). Thus as with acetylcholine, direct electrical stimulation is more potent than nervous stimulation in causing release of these hormones. In this respect also, the frog's heart differs from rabbit's heart; the latter liberates 8 times more adrenaline than noradrenaline when driven electrically (Day, 1956).

TABLE I

Adrenaline and noradrenaline released from the frog's heart as a result of stimulation of sympathetic nerves and direct electrical stimulation

Number of Experiment	Nanograms released from 1 g. of tissue per hour									
	Noradrenaline					Adrenaline				
	S	N	%+	D	%	S	N	%+	D	%
1	30	45	50	291	870	20	30	50	114	470
2	83	178	115	250	201	187	187	0	250	34
3	174	218	25	327	88	139	139	0	173	25
4	30	49	63	102	240	20	141	605	156	680
5	101	201	99	256	154	80	141	76	142	78
6	111	222	100	317	187	110	125	14	215	96
7	121	225	86	311	157	105	145	38	163	55
8	102	218	114	308	202	95	137	46	161	70
9	103	227	120	300	191	100	140	40	168	68
10	102	213	109	307	201	97	157	62	189	95
11	66	204	209	339	414	40	80	100	129	223
12	98	197	101	283	190	112	150	34	200	80
Average	93	180	94	282	203	92	131	42	172	87

S=Spontaneously beating heart.
N=Nervous stimulation.
%+=per cent. increase.
D=Direct electrical stimulation.

In the above experiments the post-ganglionic fibres of the sympathetic have been stimulated. It has also been found possible to stimulate the post-ganglionic fibres of the vagus by stimulating the white crescentic line between the sinus and the auricles. In 24 experiments, the spontaneously beating frog's heart showed no release of acetylcholine in 1 hour as found previously (Singh, Singh and Bhatnagar, 1960 *a*). Stimulation of the crescentic line by induction shocks just strong enough to produce inhibition released $0{\cdot}24 \pm 0{\cdot}1$ µg./g. wt. of acetylcholine in the next hour. Direct stimulation in the third hour caused much greater release of acetylcholine, $1{\cdot}1 \pm 0{\cdot}3$ µg./g. wt. When the crescentic line is stimulated the current is bound to spread to the muscle fibres, so that the release of acetylcholine by neural tissues is much less than indicated by the above figures. Some of the hearts analysed contained high acetylcholine content, 11–22 µg./g. wt. Thus the result of stimulating the pre-ganglionic and post-ganglionic fibres of the vagus are identical. The heart of *Rana tigrina* can be acclimatised to eserinised Ringer's solution containing 1 in 1,000 acetylcholine. After preliminary inhibition the heart recovers, the recovery being hastened by eserine. Stimulation of the vagus, when the heart is beating after recovery, produces complete inhibition, suggesting that the inhibition by vagus could not be due to release of extracellular acetylcholine.

Discussion

In these experiments it has been presumed that prolonged stimulation fatigues the sympathetic and vagal nerve-endings. This is shown both by estimating the amount of acetylcholine, adrenaline and noradrenaline released on prolonged stimulation and by recording the mechanical response. This has been studied on 3 tissues, frog's stomach muscle, dog's stomach muscle and frog's heart. The output of acetylcholine on prolonged stimulation of the vagus nerve to frog's stomach muscle and dog's stomach muscle declines in the second hour (Singh, Sharma and Bhatnagar, 1959); the output of acetylcholine (Singh, Singh and Bhatnagar, 1960 *a*), adrenaline and noradrenaline, similarly declines in the frog's heart on stimulation of the vago-sympathetic nerves. In mammalian heart also, the output of acetylcholine declines (Öberink and Essex, 1953).

The mechanical response of the above structures also declines on prolonged nervous stimulation. If frog's stomach muscle is stimulated through its nerves, the response subsides in about 2 to 3 min. though the nerves are being continuously stimulated. After subsidence of the response to nervous stimulation, direct electrical stimulation produces a contraction as large as that produced by nervous stimulation (Singh and Singh, 1947, 1948).

There is some difference between the response to nervous and direct stimulation; the former has a long latent period of about 5 to 20 sec. and the latter has a latent period about a fifth of this or less. Such a long latent period is not known in ganglion cells, so that it must be that of the nerve-endings. This difference suggests that the muscle fibres have a lesser latent period than nerve-endings and are capable of being stimulated directly. This unstriated muscle is therefore electrically excitable. Another interesting feature about this muscle is that it may not respond to acetylcholine, but yet respond to nervous stimulation, suggesting that this substance is not the transmitter at the neuromuscular junction. It is known that the stomach muscle responds to nervous stimulation after atropine. Thus an atropine-effect may occur naturally. The frog's stomach muscle is further interesting, in that 1 in 100,000 atropine improves the response to nervous stimulation, but abolishes that to acetylcholine. Eserine, 1 in 10 million, may completely inhibit the response to acetylcholine. Similarly, small concentrations of adrenaline improve the response to nervous stimulation, but the response to acetylcholine may be completely abolished.

If the dog's stomach muscle is stimulated through the vagus nerve, the mechanical response subsides in 15 to 30 min., but direct electrical stimulation then produces a powerful response. If the vagus nerve to frog's heart is stimulated, the inhibition may last about 5 min. These results are at variance with those of some other workers. Orias (1932) stimulated the pre-ganglionic fibres of the cervical sympathetic 10 times a sec. for one hour and found no evidence of fatigue in the responses of the nictitating membrane. Dye (1935) also found no evidence of exhaustion after stimulation of the pre-ganglionic nerves for 3 hours.

If the vagus nerve to the frog's heart and stomach muscle and dog's stomach muscle is stimulated it is possible that the subsidence of inhibition in the heart and the mechanical response in the stomach may be due to ganglionic block, though this is unlikely. But when the sympathetic fibres to the heart are stimulated, then there is no question of any ganglionic block, as they are post-ganglionic fibres. After fatigue of the sympathetic nerve-endings, direct stimulation produces a greater output adrenaline and noradrenaline. These, therefore, originate from the muscle cells. This is further shown by the fact that the amount of adrenaline and noradrenaline released on direct stimulation is greater than maximal stimulation of nerves. These experiments do not exclude the release of these hormones from the nerve-endings. Nervous stimulation increases the output of adrenaline as well as noradrenaline, though the increase in the output of the latter is greater

(94 per cent.) than that of the former (42 per cent.). Direct electrical stimulation causes much greater increase in output of noradrenaline (203 per cent.) than adrenaline (87 per cent.). This suggests that noradrenaline can originate from muscle cells, and adrenaline from nerve-endings.

Summary

1. Frog's heart (*Rana tigrina*) contains more noradrenaline than adrenaline.

2. Spontaneously beating frog's heart releases adrenaline and noradrenaline.

3. Stimulation of the sympathetic nerves releases adrenaline as well as noradrenaline.

4. After fatigue of the sympathetic nerves by maximal stimulation for one hour, direct electrical stimulation releases adrenaline and noradrenaline in amounts greater than can be released by nervous stimulation. These hormones therefore can originate from muscle cells.

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CONTRACTION OF UNSTRIATED MUSCLE WITHOUT FISSION OF ADENOSINETRIPHOSPHATE OR CREATINE PHOSPHATE

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That the enzymatic splitting of the terminal phosphate group of adenosine triphosphate (ATP) provides the energy for immediate utilisation in muscular contraction is yet to be proved. Fleckenstein *et al.* (1954 *a*, *b*) found no evidence for the breakdown of ATP or phosphocreatine after 1–2 second tetanus of the frog's rectus abdominus at 0° C. Similarly Mommaerts (1955) found no evidence for the fission of ATP or phosphocreatine during single twitches of turtle muscle. Dixon and Sacks (1958) found no change of ATP and adenosinediphosphate (ADP) as a result of 30 second tetanus of the cat gastroenemius muscle, under essentially anaerobic conditions. Tracer data obtained indicated that there is no redistribution of phosphate between the terminal phosphate of ATP and phosphocreatine, ruling out the possibility of resynthesis of ATP by transfer of the phosphate group of phosphocreatine.

The smooth muscle of the stomach of the frog is peculiar in that the glycerinated muscle does not respond to ATP with any contraction (Singh, 1958). Some preliminary experiments showed that this muscle presents some advantages in the study of fission of ATP and phosphocreatine during contraction. The present research was therefore undertaken to study this problem on this muscle.

Experimental

The experiments were performed on transverse pieces of stomach muscle of the frog *Rana tigrina*, both at room temperature and at 0° C. After dissection and removal of the mucous membrane the muscle was cut into two pieces. One piece was suspended in a beaker containing frog saline (Singh, 1939), but buffered with bicarbonate, pH 8. The other was left unsuspended in the same beaker and both aerated for one hour, so as to enable them to reach a stable state. The unsuspended piece gradually shortens to a constant length owing to its viscoelastic properties (Winton, 1930). This shortening is not accompanied by any expenditure of energy, as is well known in unstriated muscle. It is also known that

stretching increases the excitability of unstriated muscle, so that the shortened muscle becomes in-excitable. The muscle therefore serves admirably as a control, as it does not contract on sudden cooling or when immersed in cooled trichloroacetic acid. Further such drastic procedures, such as immersion in liquid air or liquid isopentane (Mommaerts and Schilling, 1955) to stop the chemical changes were not found necessary, as results showing, absence of fission of ATP or phosphocreatine were obtained by mere immersion in trichloroacetic acid cooled to 0° C. The contraction of unstriated muscle is known to be slow when compared with that of striated muscle, and it appears that the chemical processes are also slower, thus eliminating the use of liquid air or liquid isopentane.

The suspended piece was stimulated with isotonic potassium chloride (0·112 M KCl) for 10–30 seconds, and the other served as a control. After the attainment of contraction, the stimulated and the control muscles were quickly placed in precooled trichloroacetic acid (— 2° C.). No contraction occurred in the controls when immersed in the cold acid; rather they began to lengthen. This lengthening is due to denaturation of the contractile proteins by the acid; the unfolding of the proteins due to denaturation causes active relaxation, the process of relaxation and denaturation being similar, in that, both being due to unfolding of the contractile proteins (Singh and Singh, 1954 *a*, *b*, *c*; 1955 *a*, *b*).

The contraction of the unstriated muscle takes about 50–60 seconds. The rising phase takes no less than 10 seconds and the latent period is about 5 seconds at 18° C. (Dubuisson, 1954). As such this slow muscle can be fixed in a state of contraction with the cold acid itself.

To determine the phosphate fractious the tissue was ground in a cold mortar with the cold trichloroacetic acid solution at 0° C. and centrifuged. The acid was removed by ether extraction (thrice). Creatine phosphate and inorganic phosphate were determined by a modification of the Ennor and Stocken method (Hawk, 1954). The sample was chromatographed on paper using the solvent system of Krebs and Hems (1953). After about 24 hours, the paper was removed and the solvent allowed to evaporate under a fan. The phosphates were located by spraying with the acid-molybdate reagent according to Burrows *et al.* (1952). The spots were cut out and wet ashed, as described by Eggleston and Hems (1952) and analysed according to the heating method of Bartlett (1959), but read at 660 mμ; which procedure is about thrice sensitive than the conventional Fiske and Subba Rao (1925) procedure.

The work done by the muscle was calculated from the kymographic record.

Results

The results presented in Table I indicate that in winter, there is no decrease in the concentration of phosphocreatine or ATP due to contraction induced by potassium chloride. In summer at room temperature (Table II *a*) there is breakdown of phosphocreatine, while at 0° C.; there are again no changes in the concentration (Table II *b*) of phosphocreatine, ATP or ADP. The breakdown of phosphocreatine due to the KCl stimulation at room temperature in summer, incidently proves that the method of fixing this tissue with cold acid is justified in the case of this slow muscle.

Table I

Changes in phosphate compounds in frog's stomach muscle, as a result of contraction induced by isotonic (0·112 *M*) *potassium chloride in winter* (15° *C.*)

Number of experiments 15

S.E. = Standard error of the mean = $\sqrt{\frac{\Sigma(\bar{x}-x)^2}{n(n-1)}}$

	In μ mol./gm. of fresh tissue		Significance of difference P values
	Control muscle (Ave. $\pm$ S.E.)	Stimulated muscle (Ave. $\pm$ S.E.)	
Phosphocreatine	1·68$\pm$0·19	1·65$\pm$0·19	>0·9
Inorganic phosphorus	4·75$\pm$0·027	5·37$\pm$0·30	0·2–0·1
Adenosinetriphosphate ..	1·52$\pm$0·07	1·52$\pm$0·08	..
Adenosinediphosphate ..	1·37$\pm$0·12	1·38$\pm$0·12	>0·9
Work done by muscle in gm.cm./gm. ..	0	2·19$\pm$0·41	

Discussion

These results indicate that in unstriated muscle, as in striated muscle, ATP is not the primary substance to provide energy for contraction. In summer, when the muscle is more active and frequently contracts

TABLE II *a*

Changes in phosphate compounds in frog's stomach muscle as a result of isotonic (0·112 *M*) *potassium chloride contraction in summer* (*room temperature* 32–35° *C.*)

Numoer of experiments 4

	In μ mol./gm. of fresh tissue		Significance of difference P values
	Control muscle (Ave. ± S.E.)	Stimulated muscle (Ave. ± S.E.)	
Phosphocreatine	2·17±0·35	1·51±0·33	0·1–0·2
Inorganic phosphorus	5·00±0·52	5·7 ±0·48	0·4–0·3
Adenosinetriphosphate	1·68±0·11	1·67±0·10	0·9–0·8
Adenosine diphosphate	1·56±0·10	1·53±0·10	0·8–0·7
Work done by the muscle in gm.cm./gm.	0	7·66±0·62	..

TABLE II *b*

Changes in phosphate compounds in frog's stomach muscle as a result of isotonic (0·112 *M*) *potassium chloride in summer muscles at* 0° *C.*

Number of experiments 4.

	In μ mol./gm. of fresh tissue		Significance of difference P values
	Control muscle (Ave. ± S.E.)	Stimulated muscle (Ave. ± S.E.)	
Phosphocreatine	2·06±0·40	2·12±0·46	>0·9
Inorganic phosphorus	4·80±·030	5·25±0·50	0·5–0·4
Adenosinetriphosphate	1·61±0·16	1·60±0·18	>0·9
Adenosinediphosphate	1·43±0·28	1·40±0·34	>0·9
Work done by muscle in gm.cm./gm.	0	1·09±0·18	..

spontaneously, the diminution of the phosphocreatine content, shows that the breakdown of ATP occurs at some intermediate stage. It appears that in unstriated muscle both the mechanical and chemical changes are slow. The slowness of the mechanical change appears to be due to the physical properties of the muscle, such as viscosity. The chemical changes therefore follow the physical changes, indicating that relaxation is active.

SUMMARY

1. The fission of ATP and phosphocreatine in frog's stomach muscle has been studied.

2. In winter, there is no decrease in the concentration of ATP or phosphocreatine due to contraction induced by potassium chloride.

3. In summer, when the muscle is more active and shows frequent spontaneous contractions, there is breakdown of phosphocreatine, but if these muscles are cooled to 0° C.; then there is no change in phosphocreatine content.

4. These experiments suggest that ATP is not the primary substance that supplies energy for contraction in unstriated muscle.

We wish to thank the Indian Council of Medical Research for the generous financial grant.

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A NEW GENUS OF THE LOPHOTHALIEÆ FROM INDIA*

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(Communicated by Dr. S. Jones, F.A.SC.)

The alga which is described here was collected from the Pearl oyster beds off Tuticorin during January and March 1959. This alga was common on the shells of pearl oysters collected from the Karuval Paars (both Karai and Velangu Karuvals), 14–18 metres deep.

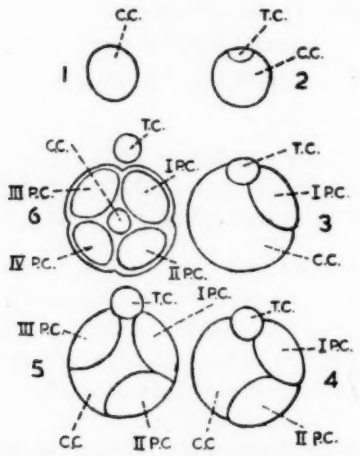
The plants, purple brown in colour, were growing on the shells of pearl oysters and attached by means of unbranched rhizoids, which were anchored to sand particles on the shells by disc-like tips. These rhizoids are formed exogenously by the pericentral cells of the older regions of the prostrate portions of the plants. They are often three-celled (Plate II, Fig. 7), rarely two- to five-celled. The erect portion arising from the prostrate portions attains a height of 4–7 cm. Superficially they resemble a *Polysiphonia* except for a dense clothing of persistent, coloured, lateral trichoblasts (Plate I, Fig. 1 and Plate II, Fig. 4).

The alga grows by an apical cell, 13–15 μ long and 8–10 μ broad (Plate I, Fig. 3). This apical cell cuts off the trichoblast initial and four pericentral cells. The order of formation of the pericentral cells is indicated in Text-Figs. 1–6. The fully developed axis consists of a central cell 22–25 μ diameter and surrounded by four pericentral cells. The diameter of the older region is 180–210 μ and the length of a segment is 113–145 μ. Cortex is absent.

The lateral branches are persistent and monosiphonous. They arise from near the top of the central cells (Plate II, Fig. 6 and Text-Figs. 7 and 8). These lateral branches are arranged spirally on the axis, anticlockwise, every fifth lateral branch coming in a line on the same side as the first, *i.e.*, $\frac{1}{4}$ spiral. Their formation in relation to the axis is indicated in Text-Figs. 7–14. A well-developed lateral branch measures about 8–12 mm. and consists of two (rarely three) small quadratic or slightly cylindrical cells at the base, a

* Published with the permission of the Chief Research Officer, Central Marine Fisheries Research Station.

very large flabellate cell in the middle, a small cell above and terminated by two very small cells, the distal of which being mucronate. Sooner or later the small basal cells shrink to form a neck connecting the trichoblast with the main axis. Even from the very early stages the flabellate cell is easily distinguishable (Plate I, Fig. 3). At the broadest part, these trichoblasts are 97–105 μ broad.



TEXT-FIGS. 1–6 (Diagrammatic): The order of formation of the pericentral cells and the position of the trichoblast cell in relation to the pericentrals.

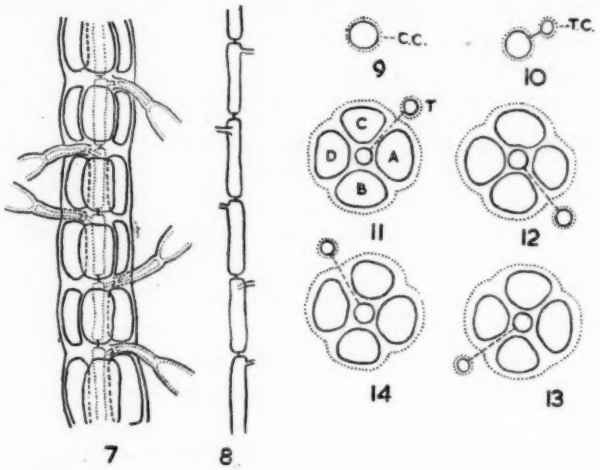
C.C., central cell; T.C., Trichoblast initial; P.C., pericentral cell (I, II, III, IV representing the pericentral cells in the order of formation.

Most of the plants collected were without fruit bodies or had only tetrasporangia. The development of the tetrasporangia is similar to that described by Iyengar and Balakrishnan (1950) for *Polysiphonia platycarpa* Boergesen. Tetrasporangia are arranged spirally on the terminal regions of the branches and of the main axis (Plate I, Fig. 2). During their formation a basal sterile cell and two cover cells are differentiated.

Only one specimen with two cystocarps was found in the present set of collections (Plate II, Fig. 5). But unfortunately, the material got damaged while cutting sections and hence details of the cystocarp formation could not be followed. As this material is not available either as a herbarium specimen or as a permanent mount on slide, Plate II, Fig. 5 may be considered as a paratype. No antheridial plant was met with in the collections.

This alga belongs to the Rhodomelaceæ and comes under the sub-family Lophothalicæ or the Lophothalia group of Kylin (1956). In the absence of

specialized stichidial branches, in the presence of four pericentral cells, in the absence of cortication and in the formation of persistent coloured monosiphonous lateral branches, the present alga comes very close to *Veleroa* of Dawson (1944). The latter is monotypic and is based on *V. subulata* Dawson. Only tetrasporic plants were recorded by Dawson. It differs markedly in the characteristic flabellate shape of the unbranched laterals (trichoblasts).



TEXT-FIGS. 7–14 (Diagrammatic): Fig. 7. Schematic representation of the thallus showing the relative positions of the axial cells, pericentral cells and trichoblasts in a fully mature part. Fig. 8. The origin of the trichoblasts from about the top of the central cells. Figs. 9–14. Represent the spiral formation of the trichoblasts, ¼ spiral arrangement; each figure representing a successive segment from top downward.

(C.C., central cell; T.C., trichoblast cell.

The lateral branches in *Veleroa*, as is understood on the basis of *V. subulata*, are many-celled, narrow and gradually tapering, all the cells being of uniform size and shape except the apical cell which is conical. Such characteristic flabellate lateral branches, as observed in my material, are not known in any other genus. These lateral branches bear a distant resemblance to the figures of *Gonctogenia subulata* J. Ag., given in Kylin (1956, p. 513, Fig. 406 C), but the flabellate cells in *Gonatogenia* are spermatangial stichidia. Further, *Gonatogenia* has five pericentral cells in the polysiphonous regions and in many other respects also it differs from the present alga. The present alga is therefore considered as a new genus and is named *Lynkiella*, after Professor H. Kylin. The specific name *Karuvalensis* indicates the locality (*Karuval paars*) from where the alga was collected.

Diagnosis.—In English: *Lynkiella* Varma gen. nov.

Thallus purple brown in colour, polysiphonous with a central axial cell and four pericentral cells, monopodial, attached to the substratum by means of 2–5 celled rhizoids produced exogenously from the older regions; bearing a dense clothing of persistent coloured 6–7 celled long determinate lateral branches (trichoblasts), middle cells of lateral determinate branches very prominently enlarged, many times longer than broad, flabellate. Tetrasporangia formed spirally on the polysiphonous axis, one sporangium per segment, development of sporangia as in *Polysiphonia*. Cystocarpic plants known. Male plants not known.

Type: *Lynkiella karuvalensis* Varma sp. nov.

Lynkiella karuvalensis Varma sp. nov.

Plants 4–7 cm. tall, attached to calcarious substrata and sand particles by means of rhizoids. Main axis in fully developed regions is 180–210 μ thick; the length of a segment being 113–145 μ. Trichoblasts arranged spirally, anticlockwise, $\frac{1}{4}$ spiral, 97–105 μ broad and 8–12 mm. long.

Type specimen collected on the shells of pearl oysters at Karai Karuval Paars near Tuticorin on 21–1–1959 and deposited in the C.M.F.R.S. Herbarium, RPV. 1038; Paratype collected at Velangu Karuval Paars on 11–3–1959 and deposited in the C.M.F.R.S. Herbarium, RPV. 1038–1, RPV. 1038–2 and RPV. 1038–3.

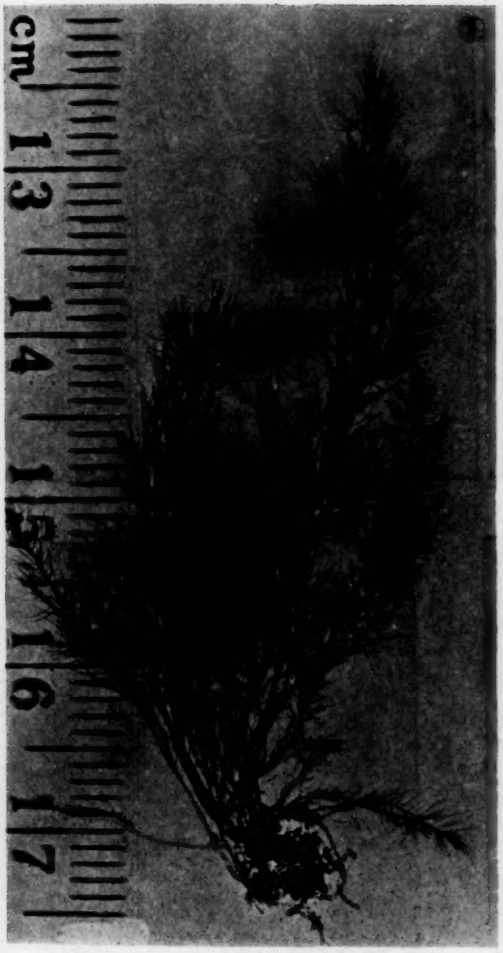
Diagnosis.—In Latin: *Lynkiella* Varma sp. nov.

Thallus purpureo-brunneus, polysiphonus, ornatus una cellula centrali et axiali et quadruplici cellula pericentrali, monopodialis, substrato fixus per rhizoideas binas-quinasve exogene productas in partibus maturioribus; thallus dense vestitus ramulis perstantibus coloratis 6–7 cellulas longis, determinatis, lateralibus (trichoblastis); cellulæ mediæ ramulorum lateralium determinatorum eminentissime dilatatæ, multo longiores quam latæ, flabellatæ. Tetrasporangia spiraliter formata insidentia axi principali polysiphono, singula singulis segmentis insidentia, evoluta ut in *Polysiphonia*. Plantæ cystocarpicæ cognitæ; plantæ masculinæ ignotæ.

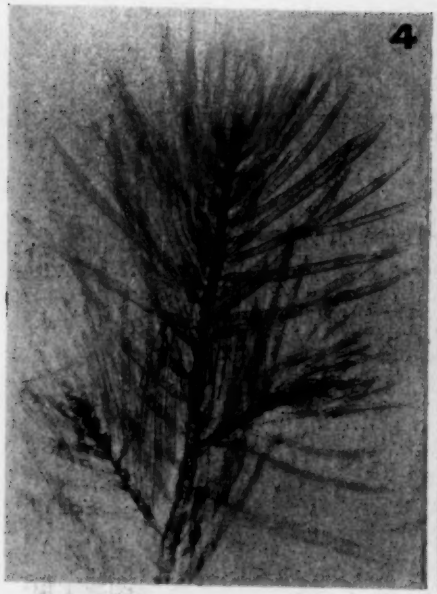
Typus: species sequens.

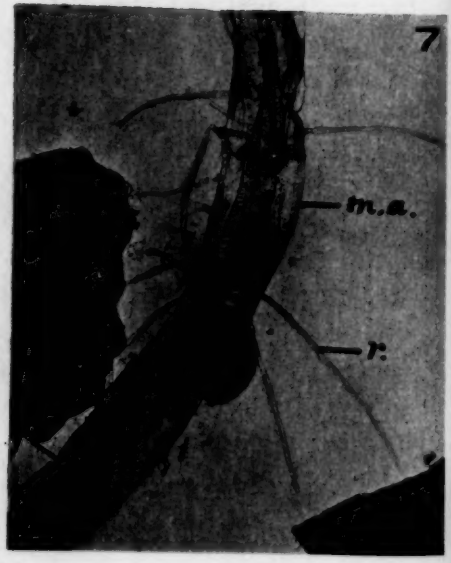
Lynkiella karuvalensis Varma sp. nov.

Plantæ 4–7 cm. altæ, fixæ substrato calcareo et particulis arenosis per rhizoideas. Axis principalis, cum plene evolutus, 180–210 μ crassus; segmenta 113–145 μ longa; trichoblasta spiraliter dispodita contra horologii manus moventia, $\frac{1}{4}$ spiralia, 97–105 μ lata, 8–12 mm. longa.



FIGS. 1–3





FIGS. 4–7

Typus lectus ostrearum conchis fixus ad Karai Karuval Paars prope Tuticorin die 21 januarii anni 1959 et positus in C.M.F.R.S. Herbario, sub numero RPV. 1038; paratypus lectus ad Velangu Karuval Paars die 11 mensis matii anni 1959 et positus in eodem herbario sub numeris RPV. 1038–1, RPV. 1038–2, RPV. 1038–3.

I am very grateful to Dr. S. Jones for the kind interest he has shown in this work. My thanks are also due to Dr. T. V. Desikachari for his very valuable criticisms and suggestions and to Rev. Father Dr. H. Santapau for kindly translating the diagnosis of the new genus and species into Latin.

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Explanation of Plates

Plate I

Fig. 1. Entire plant, ×3·4.

Fig. 2. A tetrasporic branch showing the spiral formation of tetrasporangia, ×75.

Fig. 3. Apex of the main axis showing the apical cell and a few trichoblasts, ×255. (*a.c.*, apical cell; *Tr.*, Trichoblast).

Plate II

Fig. 4. A portion of the plant showing details of the axis and the persistent trichoblasts, ×13.

Fig. 5. The cystocarpic branch with two cystocarps (Paratype), ×26.

Fig. 6. Three segments of the axis showing the origin of the trichoblasts and their relation to the pericentral cells, ×193.

Fig. 7. An older portion of the thallus showing the rhizoids arising from the pericentral cells, ×70.

(*c.*, Cystocarp; *c.c.*, Central cell; *p.c.*, Pericentral cell; *Tr.*, Trichoblast; *m.a.*, main axis of the prostrate system; *r.*, Rhizoid).

CHÆTOTAXY OF THE TERMITE, *ODONTOTERMES ASSMUTHI* HOLMGREN (ISOPTERA : TERMITIDÆ)

Part I. Soldier

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I. INTRODUCTION

THE author (Kushwaha, 1959 ; 1960 *c*) has already made a detailed study of the *chætotaxy* (*i.e.*, the system of arrangement of the differentiated bristles or macrochætæ on the various body-parts) of all the castes, *viz.*, soldier, worker

and alate, of the common mound-building termite, *Odontotermes* (*Odontotermes*) *obesus* (Rambur) and of the soldier caste of *Odontotermes* (*Odontotermes*) *horni* (Wasmann) (Isoptera, family Termitidæ), and has discussed the taxonomic differentiation of these species on the basis of the distribution of bristles, particularly on the labrum, labium and the thoracic tergites. The present account deals with the chætotaxy of the soldier caste of a third species, namely, *Odontotermes* (*Odontotermes*) *assmuthi* Holmgren, which appears to differ from the above two species in the arrangement of bristles, especially on the labrum and the thoracic tergites.

For details of the nomenclature used in these studies, please see Kushwaha (1960 *c*).

II. Material and Methods

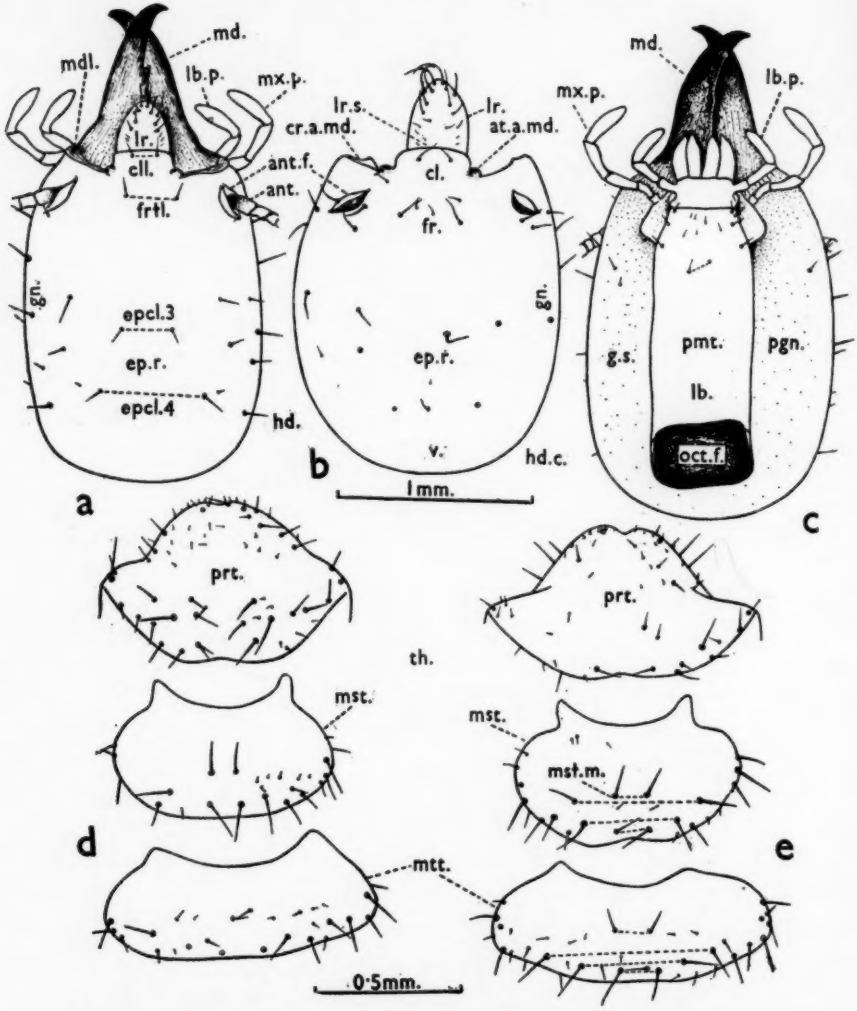
The material for the present study was kindly provided by Dr. M. L. Roonwal, Director, Zoological Survey of India, Calcutta. The exact location of the bristles as well as their general distribution on the various body-parts was studied under high magnification from permanent preparations made on slides. All the diagrams were drawn under camera lucida. In case any bristle was found missing, leaving the disc-like base behind, the latter was taken into account according to its size to put in a bristle of appropriate size in that area. Arabic numerals have been used to denominate various labral bristles, but in most cases the bristles have been named in accordance with their location on various parts of the body.

III. Head

(*a*) *Chætotaxy of the head-capsule* (Text-Fig. 1, *a–c*).—Dorsally, the head-capsule is sparsely bristled ; the *clypeal* (*cll.*) and the *frontal* (*frtl.*) pairs of bristles show bilateral symmetry and are located in the same relative position as noted in *O. obesus* and *O. horni*. All the other cranial pairs of bristles, in the present species, seem to be distributed more irregularly than in the above two species.

Ventrally, the areas are void of bristles as in the above two species (Text-Figs. 4 and 5).

(*b*) *Chætotaxy of the head-appendages* (Text-Fig. 2, *a–c* and *f–i*).—The head-appendages in *O. assmuthi* show a considerable similarity in the arrangement of bristles to that of the other two species except in the case of the labrum, where the conspicuously smaller number of pairs of labral bristles serves to distinguish it from both of them.



TEXT-FIG. 1. *Odontotermes assmuthi* Holmgren, soldier caste (cranium and thorax). (*a* and *b*) Dorsal view of the cranium, showing irregular distribution of bristles except for a few regular pairs joined by dotted lines; rounded dots indicate only the bases of bristles. (*c*) Ventral view of the cranium, showing *postmental* and *medio-postmental* bristles; postgenal areas show only a few bristles. Prementum and palpi, etc., are thickly bristled but the bristles have not been shown here. (*d* and *e*) Pro-, meso- and metanotum, showing distribution of bristles; only meso- and metanotum show some paired bristles (joined by dotted lines). It may be noted that the *mesonoto-medial* (*mst.m.*) pair is rather conspicuously located.

The *labrum* (*lr.*) in the present species has been found to bear not more than 6 or 7 pairs of labral bristles including the *apical pair* (*ap.*), whereas in *O. obesus* the number varies from 8 to 12 pairs (*vide* Table I), and in *O. horni* not less than 7 pairs have been found in every individual examined. Bilateral symmetry has been observed in all the three species discussed so far (Text-Figs. 4 and 5).

TABLE I

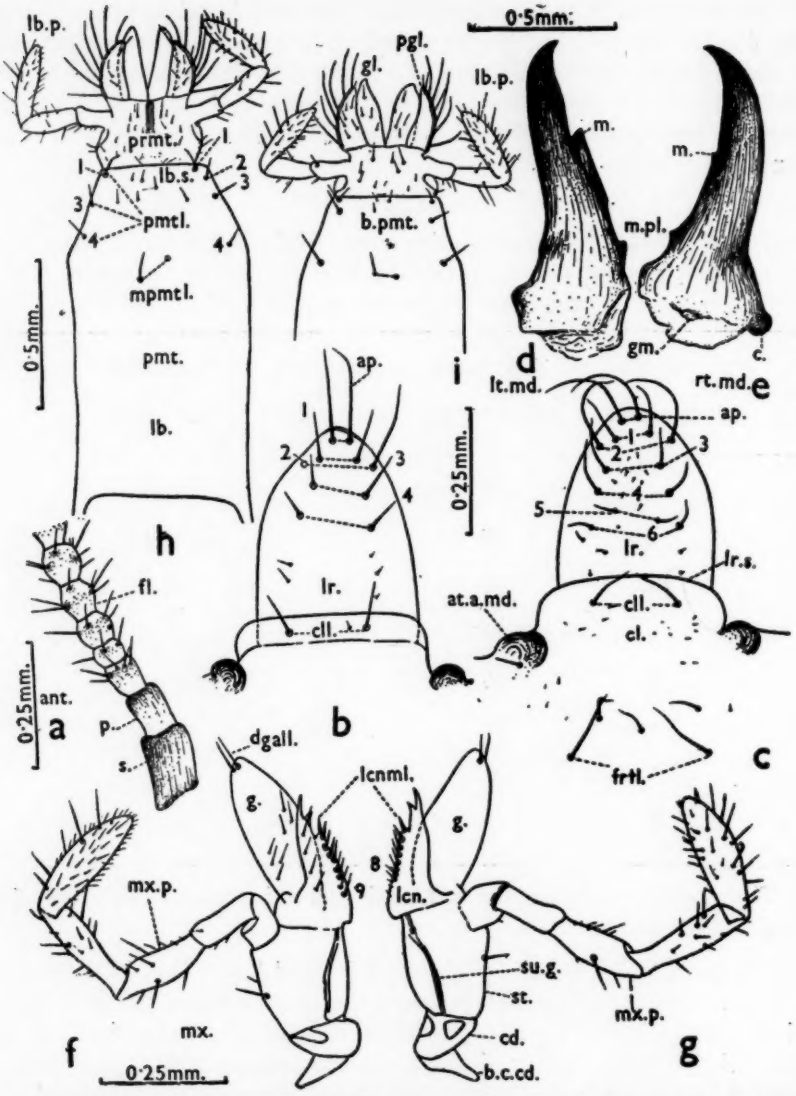
Percentage of individuals of the soldier caste of Odontotermes (Odontotermes) obesus (*Rambur*), *showing total count of bristles on the labrum. Based on* 40 *specimens examined*

Sl. No. of categories	Total count of labral bristles	Number of the individuals	Percentage of the individuals
1	16	1	2·5
2	17	1	2·5
3	18	12	30
4	19	8	20
5	20	12	30
6	21	3	7·5
7	22	2	5
8	23	1	2·5

The *scape* (*s.*) of the *antenna* (*ant.*) is devoid of bristles, as also observed in the other two species.

The characteristic bristles on the *galea* (*g.*) and *lacinia* (*lcn.*) of the *maxilla* (*mx.*) in the present species also present a similar arrangement as in *O. obesus* and *O. horni*. There is a pair of prominent *disto-galeal bristles* (*dgall.*) on the galea and the lacinia is fringed along its inner margin with 8 or 9 *lacinio-marginal bristles* (*lcnml.*). In the other two species, on the other hand, the total number of bristles varies from 9 to 11 or 12 (Text-Figs. 4 and 5).

The *labium* (*lb.*) in the present species bears about 3 or 4 *postmental pairs of bristles* (*pmtl.* 1, *pmtl.* 2, *pmtl.* 3, *pmtl.* 4) in addition to a *medio-postmental pair* (*mpmtl.*) ; all these are located more or less in the same relative



TEXT-FIG. 2. *Odontotermes assmuthi* Holmgren, soldier caste (antenna and mouth-parts). (*a*) Left antenna, showing no bristles on the *scape* (*s.*) and *pedicel* (*p.*). (*b* and *c*) Labrum together with clypeus (also frontal area in *c*), showing regular bilaterally symmetrical arrangement of bristles. (*d* and *e*) Left and right mandibles respectively. (*f* and *g*) Maxillæ, showing *disto-galeal pair of bristles* (*dgall.*) on the galea, and the lacinia showing 9 and 8 *lacinio-marginal bristles* (*lcnml.*) respectively. (*h*) Labium, showing 3 or 4 *postmental* (*pmtl.*) bristles on either side together with *medio-postmental pair* (*mpmtl.*). (*i*) Part of labium, showing *postmental* and *medio-postmental* bristles.

position as in *O. obesus*. Thus *O. assmuthi* more or less resembles *O. obesus*, both in the total number of *postmental pairs* as well as in their location. *O. horni*, on the other hand, carries a larger number of bristles—there being up to 7 or 8 *postmental bristles* located latero-marginally on each side in addition to the *medio-postmental pair*—though except in the case of a few anterior ones, no bilateral symmetry is noticeable (Text-Figs. 4 and 5).

IV. Thorax

(*a*) *Chætotaxy of the thoracic tergites* (Text-Fig. 1, *d* and *e*).—In the present species most of the bristles are large and distributed more or less regularly, particularly on the mesonotum and the metanotum.

The *pronotum* (*prt.*) carries bristles which are distributed irregularly, whereas the *mesonotum* (*mst.*) and the *metanotum* (*mtt.*) show large bristles, located mostly on the posterior margin, of which pairs showing bilateral symmetry have been joined together by dotted lines. No particular nomenclature has been assigned to these symmetrical pairs so as to obviate confusion with certain characteristic bristles known to be present on these sclerites in other species. Significant amongst the mesonotal bristles is a medial bilaterally symmetrical pair of bristles—the *mesonoto-medial* (*mst.m.*). No such distinct pair has been observed, in the other two species. Though the *mesonoto-posterior pair* (*mst.p.*) in *O. obesus* is located more or less in a similar position, the bristles, in that species, are situated wide apart, whereas in the present case they are closer together and thus occupy a medial position on the sclerite.

Differing distinctly from *O. assmuthi*, *O. obesus* carries 3 pairs of distinctly large and characteristically located bristles on the pronotum and 2 pairs each on the mesonotum and the metanotum (*vide* Table II). In *O. horni*, on the other hand, there is no such differentiation amongst the bristles either in size or location (Text-Figs. 4 and 5).

(*b*) *Chætotaxy of the legs* (Text-Fig. 3, *a–c*).—Fore-, middle- and hind-legs in the present species show the number of apical tibial spurs as follows:—Fore-tibia 3; middle-tibia 2 ; and hind-tibia 2. All the legs possess a pair of ventral tibial spurs (*v.tb.sr.*) but the fore-leg is, in addition, provided with a *dorsal tibial spur* (*d.tb.sr.*) also. Thus the spur formula in all the three species, namely, *O. obesus*, *O. horni* and *O. assmuthi* is 3:2:2.

V. Abdomen

(*a*) *Chætotaxy of the abdominal sclerites* (Text-Fig. 3, *d* and *e*).—The *abdominal tergites* (T. I–T. X,) and *sternites* (S. 1–S. 10) show a more or less

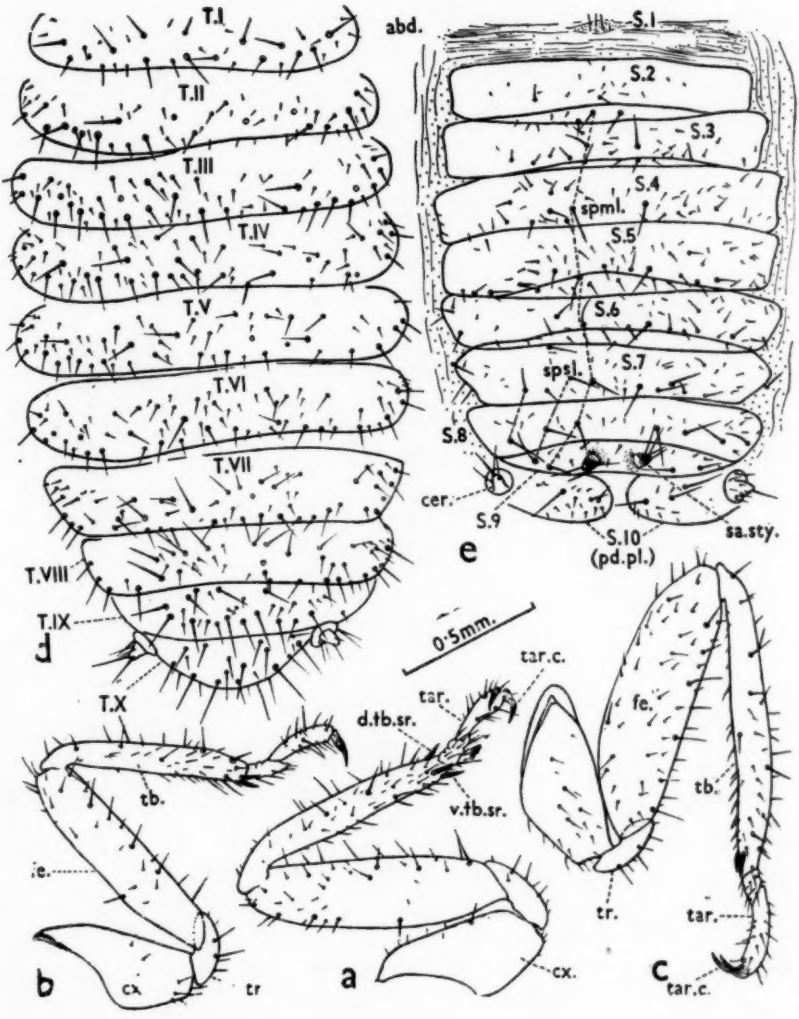
TABLE II

Characteristic differentiation between the soldier caste of three species of the genus and subgenus Odontotermes (*Isoptera*: *Termitidæ*), *in respect of the bristles on the thoracic tergites*

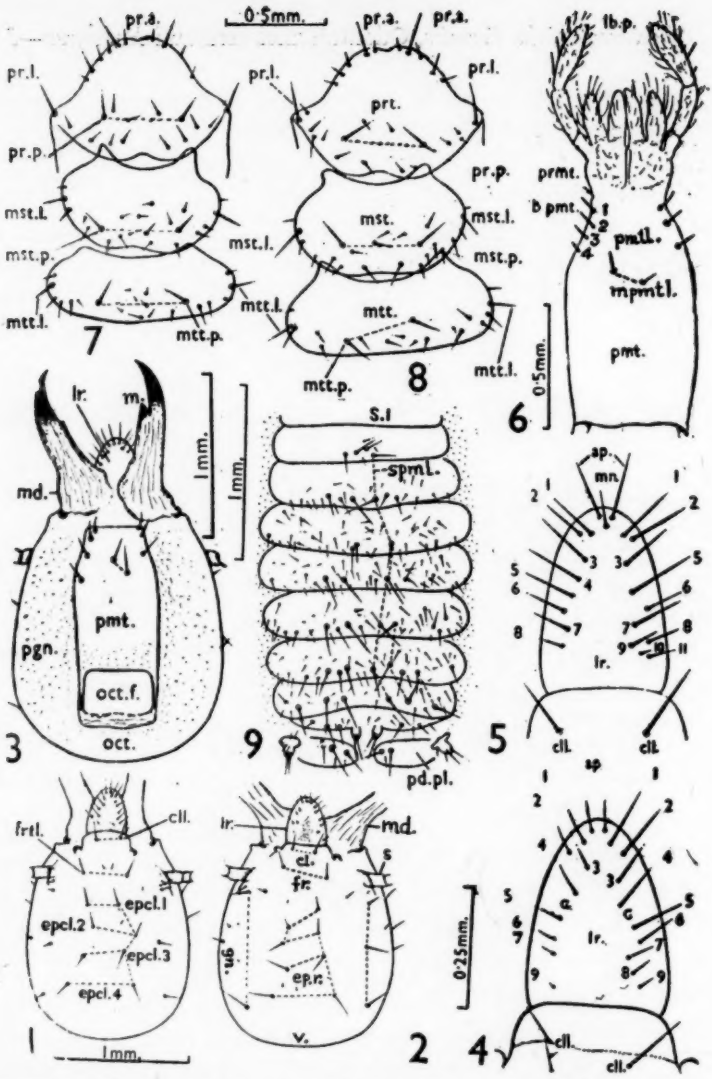
Species	Characteristic bristles on the thoracic tergites			Remarks
	Pronotum	Mesonotum	Metanotum	
O. obesus	*Pronoto-anterior* (*pr.a.*) *Pronoto-lateral* (*pr.l.*) *Pronoto-posterior* (*pr.p.*)	.. *Mesonoto-lateral* (*mst.l.*) *Mesonoto-posterior* (*mst.p.*)	.. *Metanoto-lateral* (*mtt.l.*) *Metanoto-posterior* (*mtt.p.*)	Characteristic bristles distinctly large and well differentiated from the rest.
O. horni	..	..	..	Most of the bristles uniform and undifferentiated in size
O. assmuthi	..	*Mesonoto-medial* (*mstlm.*)	..	A few more symmetrical pairs on meso- and metanotum

similar distribution of bristles as observed in both *O. obesus* and *O. horni*. The tergites are irregularly bristled, while the sternites show a bilaterally symmetrical arrangement of the larger bristles which have been named *sterno-posteromedial* (*spml.*), *sterno-posterosublateral* (*spsl.*), etc., as in the other two species (Text-Figs. 4 and 5).

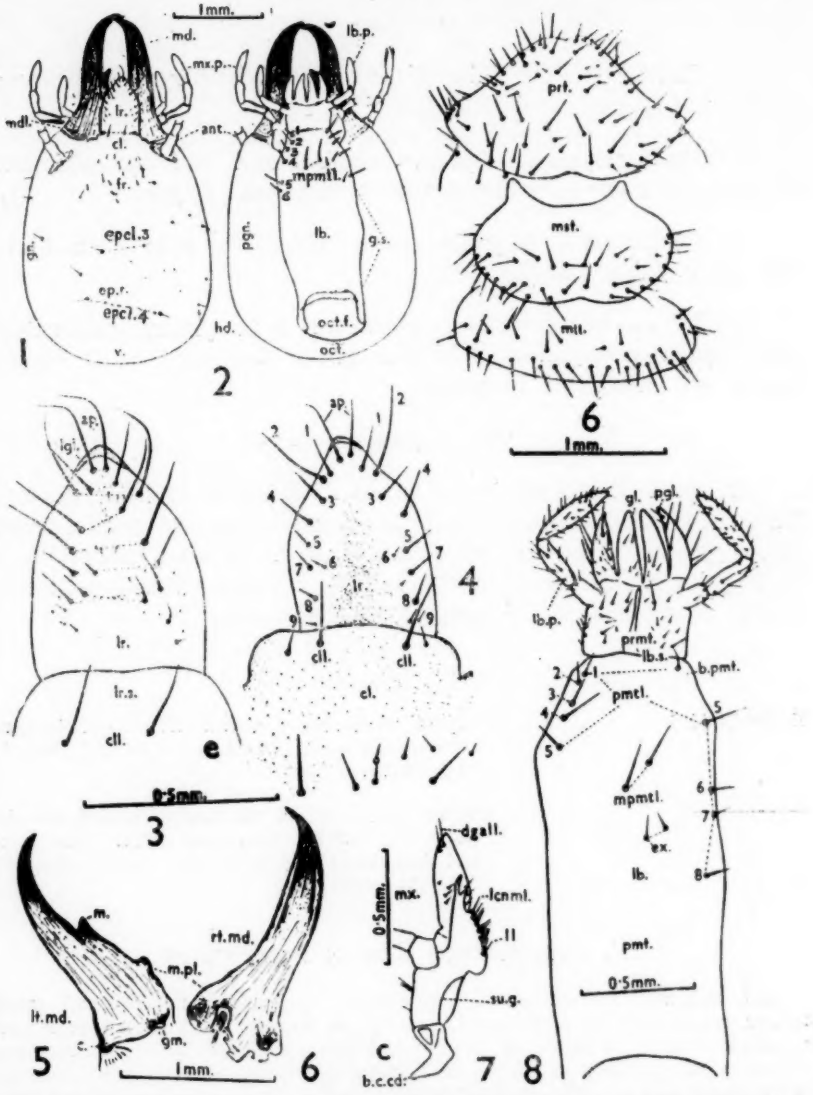
(*b*) *Chætotaxy of the abdominal appendages* (Text-Fig. 3, *d* and *e*).—The bristles on the *anal cerci* (*cer.*) and the *subanal styles* (*sa.sty.*) show no characteristic differences between the three species discussed so far.



TEXT-FIG. 3. *Odontotermes assmuthi* Holmgren, soldier caste (abdomen and legs). (*a*) Left fore-leg, showing a single *dorsal tibial spur* (*d.tb.sr.*) and a pair of *ventral tibial spurs* (*v.tb.sr.*). (*b* and *c*) Left middle- and hind-leg show only a pair of *ventral tibial spurs* (*v.tb.sr.*). (*d*) Abdominal tergites (T. I—T. X), showing irregular distribution of bristles. (*e*) Abdominal sternites (S. 1—S. 10), showing the paired *sterno-posteromedial* (*spml.*) *and sterno-posterosublateral* (*spsl.*) setæ.



TEXT-FIG. 4. *Odontotermes obesus* (Rambur), soldier caste. (1) Dorsal view of the cranium, showing nearly all symmetrical pairs of large bristles. (2) Ditto, showing all the symmetrical pairs medially but with some lateral bristles missing. (3) Ventral view of the cranium, showing lateral *postmental* (*pmtl.*) and *medio-postmental* (*mpmtl.*) bristles; the left bristle of the *fourth postmental pair* (*pmtl.* 4) being absent. (4) Labrum, together with clypeal region, showing the paired bristles except for the 8th bristle on the left which is missing; 'G' indicates gap observed between the bristles. (5) Ditto, showing a *median bristle* (*mn.*) present; right 4th, and left 9th, 10th and 11th bristles missing. (6) Labium, showing *postmental* (*pmtl.*) and *medio-postmental* (*mpmtl.*) bristles joined by dotted lines. (7 and 8) Three thoracic tergites; the pronotum showing three symmetrical pairs of large bristles, *i.e.*, *pronoto-anterior* (*pr.a.*), *pronoto-lateral* (*pr.l.*) and *pronoto-posterior* (*pr.p.*); the meso- and metanotum showing only two pairs each, *i.e.*, *mesonoto-lateral* (*mst.l.*) and *metanoto-lateral* (*mtt.l.*) (9) Abdominal sternites 1–10 (S. 1–S. 10 = *pd. pl.*) showing a row of large *sterno-posteromedial* (*spml.*), *sterno-posterosublateral* (*spsl.*) and a few other paired bristles laterally; first three rows of symmetrical bristles on the right joined by dotted lines.



TEXT-FIG. 5. *Odontotermes horni* (Wasmann), soldier caste. (1) Dorsal view of the cranium, showing an irregular distribution of bristles except for a few pairs joined by dotted lines. (2) Ventral view of the cranium, showing characteristic *postmental* and *medio-postmental* bristles; postgenal area is naked, having no bristles. The prementum and palpi, etc., are thickly bristled but the bristles not shown here. (3 and 4) Labrum together with clypeus, showing a regular bilaterally symmetrical arrangement of bristles. (5 and 6) Left and right mandibles respectively. (7) Maxilla, showing *disto-galeal pair* (*dgall.*) of bristles on the galea, and the lacinia showing 11 *lacinio-marginal bristles* (*lcnml.*). (8) Labium, showing *postmental* and *medio-postmental* (*mpmtl.*) bristles.

VI. SUMMARY

1. Chætotaxy of the soldier of the termite, *Odontotermes* (*Odontotermes*) *assmuthi* Holmgren, is described.

2. The arrangement of bristles on the head and the abdomen in *O. assmuthi* closely resembles that of *O. obesus* and *O. horni.*

3. The labrum in the present species carries fewer pairs of labral bristles than in *O. obesus* and *O. horni.*

4. The mesonotum and the metanotum in the present species show a more regular arrangement of bristles than in *O. obesus* ; in *O. horni* the bristles are distributed irregularly.

VII. ACKNOWLEDGMENTS

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IX. ABBREVIATIONS USED IN TEXT-FIGURES

abd., abdomen; ***acl.***, anteclypeus; ***ant.***, antenna; ***ap.***, apical bristle of labrum; ***at.a.md.***, anterior articulation of mandible; ***b.c.cd.***, basal condyle of cardo; ***b.pmt.***, blade of postmentum; ***c.***, condyle of cardo; ***cd.***, cardo; ***cer.***, cercus; ***cl.***, clypeus; ***cll.***, clypeal bristle; ***cr.a.md.***, cranial articulation of mandible; ***cx.***, coxa; ***dgall.***, disto-galeal bristle; ***d.tb.sr.***, dorsal tibial spur; ***epcl.*** 1, ***epcl.*** 2, ***epcl.*** 3....etc., epicranial bristles 1, 2, 3.....etc.; ***ep.r.***, epicranial region; ***ex.***, extra bristle; ***fe.***, femur; ***fl.***, flagellum; ***f.l.***, fore-leg; ***fr.***, frons; ***frtl.***, frontal bristles; ***g.***, galea; ***gl.***, glossa; ***gm.***, ginglymus; ***gn.***, gena; ***gpgl.*** 1, ***gpgl.*** 2, ***gpgl.*** 3., geno-postgenal bristles 1, 2, 3 respectively; ***g.s.***, gular suture; ***hd.***, head; ***hd.c.***, head-capsule; ***h.l.***, hind-leg; ***lb.***, labium; ***lb.p.***, labial palp; ***lb.s.***, labial suture; ***lcn.***, lacinia; ***lcnml.***, lacinio-marginal bristle; ***lgl.***, lingula (hyaline tip of labrum); ***lr.***, labrum; ***lr.s.***, labral suture; ***lt.***, left; ***m.***, marginal tooth of mandible; ***md.***, mandible; ***mdl.***, mandibularia (or trochantin of mandible); ***m.l.***, middle-leg; ***m.pl.***, molar

plate; *mpmtl.*, medio-postmental bristle; *mst.l.*, mesonoto-lateral bristle; *mst.m.*, mesonoto-medial bristle; *mst.p.*, mesonoto-posterior bristle; *mtt.*, metanotum; *mtt.l.*, metanoto-lateral bristle; *mtt.p.*, metanoto-posterior bristle; *mx.*, maxilla; *mx.p.*, maxillary palp; *oct.*, occiput; *oct.f.*, occipital foramen; *p.*, pedicel; *pcl.*, postclypeus; *pd.pl.*, podical plates; *pgl.*, paraglossa; *pgn.*, postgena; *prt.*, pronotum; *pmtl.*, postmental bristle; *pr.a.*, pronoto-anterior bristle; *prgl.*, proximo-genal bristle; *pr.l.*, pronoto-lateral bristle; *prmt.*, prementum; *pr.p.*, pronoto-posterior bristle; *rt.*, right; S. 1, S. 2,.....S. 10, sterna first, second.....tenth; *s.*, scape; *sa.sty.*, subanal styles; *st.*, stipes; T. I, T. II,.....T. X, terga first, second.....tenth; *tar.*, tarsus; *tar.c.*, tarsal claw; *tb.*, tibia; *tb.sr.*, tibial spur; *th.*, thorax; *v.*, vertex; *v.tb.sr.*, ventral tibial spur.

LIFE WITHOUT BREATHING OXYGEN

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Many attempts have been made in the therapeutic field to oxygenate the blood by routes other than the pulmonary. The only method by which any considerable quantities of oxygen that can be introduced into the blood by an extra-pulmonary route is by intravenous injection of oxygen. The amount that can be thus introduced under normal conditions is very small, about 25% of the total requirements in dogs and 5–6% in human beings. There are however two methods by which it has been found possible to supply all the oxygen requirements of dogs by the intravenous route. Singh in 1935 found that the amount of oxygen that could be injected intravenously was increased if the atmospheric pressure, under which the experiment was performed, could be raised. It is well known that the treatment of caisson disease is to put the patient, back into the caisson under increased atmospheric pressure, so that the bubbles which are released on decompression are dissolved on recompression. It follows therefore that if bubbles of gas are artificially introduced into the blood, they will dissolve if the atmospheric pressure under which the experimental animal is placed, is increased. Using this technique, Singh was able to keep a dog alive without breathing oxygen through the lungs for 16 minutes by injecting oxygen intravenously under 4 atmospheres of air pressure.

The second method is to lower the oxygen consumption by hypothermia to such an extent, that it equals the amount of oxygen that can be injected intravenously (Singh, 1960). This can be done if the body temperature is lowered to 20–22° C.; the oxygen consumption falls to 1–2 c.c. per kg. per minute, which is equal to intravenous dose of oxygen. Singh was thus able to keep 4 dogs alive without breathing oxygen through the lungs for 20 minutes.

In the present research oxygen has been injected intravenously in dogs at 24–25° C. and 27–28° C., as these temperatures are of importance in surgery under hypothermia.

Nysten (1811) was the first to inject oxygen intravenously. Using dogs, he injected 20 c.c. of oxygen per minute repeated every 3–4 minutes. A dose

of 30–40 c.c. per minute was fatal. Intravenous oxygen was next given by Demarquay (1811) in dogs. Gaertner (1902) gave 1–2 c.c. per kg. of body weight per minute to dogs. Mariani (1902) was the first to inject oxygen intravenously into a living human being. He injected 80 c.c. of oxygen in half an hour into a dying phthisical patient. Stuertz (1904) gave oxygen to a dog of 15 kg. body weight at a rate of 3 c.c. per kg. per minute for two hours. Tunnicliffe and Stebbing (1916) gave 10–20 c.c. of oxygen per minute for periods of 10–15 minutes divided by pauses of 2–3 minutes, to three patients. Bourne and Smith (1927) found the dose for dogs was 1–3 c.c. per kg. of body weight per minute. Dick (1939) found the dose for dogs to be 2–3 c.c. per kg. per minute. Singh and Shah (1940) gave 10–20 c.c. of oxygen intravenously to six human patients with success. Ziegler (1942) gave intravenous oxygen to human patients at the rate of 200–600 c.c. per hour and claimed beneficial results. Grodins, Ivy and Adler (1943) gave intravenous oxygen to 12 dogs at the rate ranging from 0·21–3·17 c.c. per kg. per minute, fatal dose in one dog being 3·2 c.c. per kg. per minute, and did not claim any beneficial results. Jacobi, Klein, Rascoff, Kogut, Auerbach and Jennings (1946) gave oxygen intravenously to dogs for 10–14 hours at the rate of 100 c.c. per hour and found great improvement in hæmorhagic shock. They also gave to three human patients at the rate of 60–600 c.c. per hour in quantities ranging from 7,000–18,000 c.c. Sanders and Isoe (1947) gave oxygen to six patients without any beneficial results. Cole (1951) gave intravenous oxygen to patients with limited success; small quantities of this gas had remarkable anti-shock properties and could be administered with relative safety.

Metabolism is reduced in hypothermia if shivering is avoided (Penrod, 1949; Bigelow, Lindsay, Harrison, Gordon and Greenwood, 1950; Dundee, Gray, Mesham and Scott, 1953; Dundee, Scott and Mesham, 1953). During hypothermia, therefore, it would be possible to provide a greater fraction of oxygen requirements by the intravenous route. In hypothermia, there are two opposing factors that affect the intravenous injection of oxygen. During hypothermia, the solubility of oxygen in the plasma is increased by about 50% (Rosenhain and Penrod, 1951). Sluggishness of circulation gives greater time for the absorption of oxygen. These two factors favour intravenous injection of oxygen. Sluggishness of circulation acts in opposite direction also, as the oxygen accumulates in the veins and does not move forward fast enough. Therefore it is expected that at a certain temperature the intravenous injection of oxygen should be maximum. This has been realised experimentally in this research.

METHODS

Unselected mongrel dogs varying in weight from 14–22 kg. were used. A preliminary injection of atropine sulphate, gr. 1/100, was given subcutaneously. Basal nacrosis was induced by intraperitoneal injection of 30 mg./kg. body weight pentobarbitone. Observations were made at 37° C., 27–28° C. and 24–25° C.; at 27–28° C., natural respiration was recorded. At 24–25° C. artificial respiration was employed. Electrocardiograms were recorded frequently to guage the condition of the heart. Oxygen was injected from a cylinder by means of a side-tube connected with a cannula (Singh, 1935). The cannula was attached to a burette filled with normal saline and was tied into a small vein of the leg. The injection was made by means of two 50 c.c. record syringes which filled and emptied alternately.

RESULTS

Intravenous oxygen at 37° *C.*—From the experiments of various workers quoted above, the intravenous dose of oxygen at normal body temperature is 1–3 c.c. per kg. per minute (Gaertner, 1–2 c.c.; Stuertz, 3 c.c.; Bourne and Smith, 1–3 c.c.; Grodins *et al.*, 1–3 c.c.). Our observations have been similar, 1–3 c.c. in dogs of 12–20 kg. body weight. Pulmonary embolism is produced by small doses, about 1 c.c., but if this is ignored, oxygen can be injected in greater amounts, up to 2–3 c.c. This is presumably due to opening up of more blood vessels as a result of blockage of some, there being overcompensation (Singh, 1936). If the heart is embarrassed due to filling with gas, the injection should be discontinued for a few minutes; the heart recovers quickly as shown by the electrocardiogram.

The oxygen consumption of dogs at 37° C. is about 6–7 c.c. per kg. per minute. It would appear that in dogs at normal atmospheric pressure about 25% of the oxygen requirements can be met by intravenous oxygen. It would therefore require injection under 4 atmospheres of air pressure to keep a dog alive without breathing oxygen through the lungs as found experimentally by Singh (1935).

Intravenous oxygen at 27–28° *C.*—In 10 dogs, weighing from 14–23 kg., the amount of oxygen that could be injected was $2 \cdot 2 \pm 0 \cdot 5$ c.c. per kg. per minute varying from 1·2–3 c.c. per kg. per minute (Table I). Thus there is no significant difference between the amount of oxygen that could be injected at 37 and 27° C. The oxygen consumption figures in Table I are calculated from Bigelow *et al.* (1950). It will be seen that from 40–86% of the oxygen requirements of the animal could be met by intravenous oxygen.

TABLE I

Intravenous injection of oxygen in dogs at 27–28° C. body temperature

No. of experiment	Weight of dog; kg.	Oxygen consumption per kg. per min.	Oxygen injected; c.c. per min.	Oxygen injected; c.c. per kg. per min. up to maximum dose	Total amount of oxygen injected c.c.	Percentage replacement of pulmonary oxygenation by intravenous route
1	14	48	10		200	
			20		400	
			40	2·8	1,200	74
2	16	54	10		200	
			20		400	
			30	1·8	1,600	62
3	14	48	10		200	
			20	1·4	1,400	40
4	17	58	10		200	
			20		400	
			40	2·3	1,800	70
5	17	58	20		200	
			40		400	
			50	3	2,400	86
6	14	48	10		200	
			20		400	
			30	1·7	1,600	62
7	23	78	20		200	
			40	1·7	1,200	49
8	20	68	20		200	
			40	2	1,200	59
9	16	54	20		200	
			40	2·5	1,200	74
10	18	61	20		200	
			40	2·2	1,200	65
Mean of maximum dose				2·1±0·5		

As there is a mild anoxæmia in hyphothermia, it has been noticed electrocardiographically, that intravenous injection of oxygen improves cardiac action.

Intravenous oxygen at 24–25° *C.*—In 9 dogs weighing from 18–22 kg., the amount of oxygen that could be injected was $2 \cdot 4 \pm 4$ c.c. per kg. body weight per minute, varying from 2·2–3 c.c. (Table II). The entire oxygen requirement of the animal could be met by intravenous oxygen. Thus at this temperature, there is a definite effect of hypothermia on the amount of

TABLE II

Intravenous injection of oxygen in dogs at 24–25° *C. body temperature*

No. of experiment	Weight of dog; kg.	Oxygen consumption; c.c. per min.	Oxygen injected; c.c. per min.	Oxygen injected; c.c. per kg. per min.	Total amount of oxygen injected c.c.	Percentage replacement of pulmonary oxygenation by intravenous route
1	18	50	40		1,100	
			50	2·8	1,100	100
2	20	55	40		220	
			50	3	2,640	100
3	20	55	40		220	
			60	3	2,500	100
4	20	55	40		200	
			60	3	8,000	100
5	18	50	20		200	
			40	2·2	4,800	81
6	20	55	60	3	4,800	100
7	22	60	60		11,400	100
8	18	50	40	2·2	600	81
9	16	40	40	2·5	3,300	100
Mean of maximum dose				2·4±4		

oxygen that can be injected intravenously. With further lowering of temperature, the circulation becomes too sluggish, and the amount of oxygen that can be injected is about 1 c.c. per kg. per minute (Singh, 1960).

Discussion

In hypothermia there are three factors which favour intravenous injection of oxygen. These are increased solubility of oxygen in the plasma, increase in the circulation time and a mild anoxæmia. These factors apppear to operate maximally at 25° C.; thereafter the sluggishness of circulation acts in the opposite direction. Much more oxygen can be injected in dogs intravenously per kg. body weight per minute than in human beings. The reason for this is probably that in experimental animals oxygen is injected under anæsthesia, and so the acute dyspnœa due to pulmonary embolism is partly counteracted. Hypothermia counteracts the dyspnœa still further, as the respiratory centre is depressed. Intravenous oxygen has always been given in conscious human beings. It is possible that greater quantity of oxygen could be injected into human beings if it is found possible to paralyse the pulmonary receptors responsible for dyspnœa.

Summary

1. Oxygen has been injected intravenously into dogs during hypothermia.

2. There was no significant difference between the amount of oxygen that could be injected at 37° C. and at 27–28° C. But at the latter temperature, owing to reduction in metabolism, about 40–70% of the oxygen requirements of the animal could be met by intravenous oxygen.

3. At 24–25° C., slightly greater quantity of oxygen could be injected than at 27–28° C., but owing to greater reduction in metabolism, the entire oxygen requirement of the animal could be met by the intravenous route.

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ECOLOGY OF *ECHINOCHLOA COLONUM* LINK.*

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Introduction

So far, floristic studies in this country have developed on two lines (Misra, 1958). Listing of species and description of morphological features of the plants based on type collections is the first line. That these floras are not adequate and appropriate samples of plant populations is evident from the frequent revisions of earlier publications. For example, Bor (1941) has shown how earlier workers have missed many diverse forms within larger aggregates, in the case of grasses.

Ecologists have been busy in the study of vegetation in relation to its environment and in this we have the second approach to floristic studies. The work of Dudgeon (1920) for the Upper Gangetic Valley and the forest types of India and Burma by Champion (1936) are noteworthy contributions in understanding the nature of vegetation in relation to environment.

A third line of approach yet to be explored for floristic studies in India, the importance of which has been stressed by Misra (1958), is based on the concept of the biological flora of a region. Ecological flora has also the same approach and aim in view, but lays special emphasis upon the ecology of distribution of the species. A more or less similar type of study of the vegetation of the chalky downs and dunes of England, with biological notes on each species has been made by Salisbury (1952).

For an understanding of the biological and ecological equipment of the species and its relation to environmental factors, ecological life-history studies have to be pursued. Importance of such life-history studies has been stressed by Pelton (1951, 1953), Tansley (1946) and Whitehead (1957). Salisbury (1928), Anonymous (1941), Pelton (1951, 1953) and Anonymous (1958) have given elaborate schemes of study of ecological life-cycle of plants. Salisbury

* Part of a thesis submitted for the Ph.D. Degree of the Banaras Hindu University.

(1928) realised the importance of autecological studies for its own sake and also for a better understanding of plant communities and perhaps these ideals lead him to put forth a scheme of the ' Biological Flora of the British Isles '. This was almost forgotten for about 12 years until it was revised and finally adopted as a co-operative venture by the British Ecological Society in 1941 (Anonymous, 1941). Since then, a large number of species have been worked out and the accounts are published in the subsequent issues of the *Journal of Ecology*.

Work in this direction with the aim of producing Biological or Ecological flora is lacking in this country, though some work on the autecology of a few species have been done (Misra and Siva Rao, 1948 ; Bakshi, 1952 ; Pandeya, 1953 ; Mall, 1956, etc.). The present author has worked on the ecology of ten common species as a preliminary contribution to the Ecological Flora of Varanasi District (Ramakrishnan, 1959).

HABIT AND HABITAT

Echinochloa colonum Link.† (*Panicum colonum* Linn.) is an annual tufted grass (culms, 15 to 60 cm.), erect, ascending or shortly creeping below. The plant grows as a weed in cultivated grounds, grasslands, water-logged soils such as along the margin of ponds and drainage channels, ditches and puddles. The plant prefers exposed areas and is rarely to be found in shaded localities.

GEOGRAPHICAL DISTRIBUTION

E. colonum occurs throughout India and Ceylon ascending the Himalayas to 6,000 ft. (Hooker, 1872 to 1897). The plant is distributed in Tropical Asia and Africa and has been introduced in Tropical America, Australia and Polynesia.§

ECOTYPIC DIFFERENTIATION

The adaptively differentiated populations, within the species or ecotypes, are very well known to modern ecologists through many studies beginning with the works of Turesson (1922 *a*, *b*, 1923, 1925, 1930). The outstanding study of Clausen *et al.* (1940, 1948) has shown, for example, the existence of chains of climatic races in a number of perennial species ranging from the sea-level to above the timberline in California.

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† The specific name 'colonum' is used here following Bor (1941); *E. colona* is used by others.

§ This information was obtained from the Director, Royal Botanic Gardens, Kew, Richmond, Surrey.

In *E. colonum*, two distinct populations have been recognised, by culture experiments, at Varanasi. These are found to be mainly related with the moisture in the substratum.

Ecotype No. 1.—The tall form, growing in very moist to water-logged soils along the margins of ponds and drainage channels (Plate III, Fig. 1).

Ecotype No. 2.—The short form growing in comparatively drier localities (Plate III, Fig. 1). In nature, this form is more frequently subjected to grazing, scraping, etc., than the former one.

The tall form is found to have longer internodes and more vigorous growth compared to the short form (Plate III, Figs. 1 and 2). A summary of field observations regarding these characters is given in Table I.

TABLE I

Measurements taken from natural population for E. colonum

Growth form	Mean height of plant (cm.)	Mean length of leaf (just below inflorescence) (cm.)	Mean length of internode (just below inflorescence) (cm.)
Tall form ..	51·35 ± 7·23	10·13 ± 3·27	9·005 ± 2·02
Short form ..	18·00 ± 5·03	4·90 ± 1·54	3·205 ± 0·99

Hall (1932) and Hiesey (1940) have stressed the importance of transplant experiments in the study of ecotypes. They are designed to give definite evidence as to the nature of certain plant characters. Accordingly, small segments (ramets) of the two forms of *E. colonum* grown in a neutral substratum in the culture garden maintained their original form when fully developed (Plate III, Fig. 2). A summary of the results of transplant experiments is given in Table II.

The transplant experiments indicate that the two forms are genetically distinct. To confirm this observation, the two populations were cultured in the neutral substratum, of the garden from seeds. A summary of the results obtained at the conclusion of the cultures is given in Table III.

The two forms are distinct in spite of the fact that they are raised from their respective seeds (Plate IV, Fig. 1). There is significant difference in the

TABLE II

Growth measurements of E. colonum *after transplantation*

Growth form	Mean length of shoot (cm.)	Mean length of leaf (just below inflorescence) (cm.)	Mean length of internode (just below inflorescence) (cm.)	Average seed output
Tall form ..	33·2±3·42	9·94±2·24	8·26±1·32	20264
Short form ..	13·2±1·15	4·14±0·55	3·00±0·71	1387

TABLE III

Growth measurements of E. colonum *after culturing the two forms from seeds*

Growth form	Mean length of shoot (cm.)	Mean length of leaf* (cm.)	Mean length of internode* (cm.)	Average fresh wt. of shoot (g.)	Average dry wt. of shoot (g.)	Average seed output
Tall form ..	64·9±7·5	12·2±1·3	10·2±2·0	65·2	16·54	10620
Short form ..	40·4±7·2	8·4±2·3	7·1±1·8	32·2	6·30	1768

* Leaves and internodes examined from just below the inflorescence.

values obtained for length of the shoot, length of the internode, seed output, etc.

A comparative study of Tables I, II and III shows that the values obtained are much greater for both the ecotypes under cultural conditions which is naturally expected. Thus, transplant studies and cultures raised from seeds of these two forms confirm the genetical distinctness of the two forms.

SIZE AND WEIGHT OF SEEDS

The seeds are broadly elliptic. The length of the seed varies from 1·922 to 2·338 mm. and the breadth varies from 1·117 to 1·403 mm. The shape index (length/breadth ratio) varies from 1·4 to 2·1.

The weight of the seeds of the tall and the short forms of *E. colonum* growing in wild and in cultures in a neutral substratum is noted. The tall form has an average seed weight of 1·37 and 1·26 mg. respectively in wild and in cultures of a neutral substratum whereas that of short form gives 1·17 and 1·19 mg. respectively in wild and in cultures. A marked difference in the seed weight of the tall and the short forms is discernible. This difference is maintained in the growths in a neutral substratum.

Environmental Factors

Edaphic

As already mentioned, moisture level in the soil controls the distribution of the two ecotypes. The tall form occurs in very moist to water-logged soils and the short form in compartively drier situations. Soils were analysed for the following.

pH.—pH of the soil was determined electrometrically using a Photovolt pH meter. The reaction of the soil is alkaline having a range of 7·2 to 8·2.

Carbonate content.—The tall form is found to grow in soils with lower carbonate content while the short form grows also in soil with larger amount of carbonate.

Exchangeable calcium.—Both the ecotypes prefer non-calcareous substratum. The tall form is found to grow in calcium-poor soils only but the short form occasionally grows in soils richer in exchangeable calcium.

Nitrate content.—Nitrate nitrogen in soil varies from 2·25 to 4·50 mg./100 g. of soil.

Organic matter.—The organic matter content in the soil varies from 1·55 to 5·20 per cent.

The detailed soil analysis data are set in Table 1V.

Performance of the Plant in Various Habitats

A definite correlation could be established between the performance of the plants as indicated by the average seed output values and the exchangeable calcium in the soil (Table V).

As already mentioned, the plant is typically a calcifuge. The seed output values for the tall form and the short form decrease with an increase in calcium in the soil ; the best performance is obtained in calcium-poor soils,

TABLE IV

Soil analysis data of E. colonum

Locality	Moisture content (%)	pH	Carbonate content (%)	Exchangeable calcium (m.e. %)	Nitrate content (mg./100 g. of soil)	Organic matter (%)	Remarks
Tall form—							
Latifshaw	28·4	7·6	0·035	8·0	2·75	3·30	Water-logged soil
University area ..	27·2	7·3	0·245	3·7	2·50	3·34	do.
Akhari	47·4	7·3	0·253	12·0	2·25	3·80	Very moist soil
Ramnagar	43·6	7·3	0·105	2·0	2·75	2·20	Water-logged soil
Sarnath	30·2	7·5	0·090	2·9	3·20	2·82	do.
Short form—							
Rajghat	14·7	8·2	1·426	20·3	2·50	5·20	Moderately moist
Ramnagar	12·8	7·8	3·980	28·0	4·50	1·55	do.
Akhari	18·2	7·5	0·286	9·0	2·75	3·60	do.
University area ..	8·9	7·6	0·942	12·4	2·50	2·22	do.
Sarnath	10·5	7·2	0·160	6·5	2·70	2·36	do.

TABLE V

Performance of E. colonum *in various habitats*

Locality	Average height of plant (cm.)	Average seed output	Exchangeable calcium in soil (m.e. %×)
Tall form—			
Latifshaw	36·0	1842	8·0
University area ..	60·0	3787	3·7
Akhari	58·0	1033	12·0
Ramnagar	37·7	3888	2·0
Sarnath	44·5	3624	2·9
Short form—			
Rajghat	15·0	481	20·3
Ramnagar	19·5	345	28·0
Akhari	23·5	595	9·0
University area ..	25·0	498	12·4
Sarnath	22·5	788	6·5

In general, the tall form has a much higher seed output value than the short form. The seed output value in cultures is much higher for the two ecotypes than in wild. Plants which are frequently subjected to biotic influences like grazing and scraping show poorer growth and hence lower seed output.

No correlation, however, could be established for other factors like pH, carbonate, nitrate and organic matter in the soil.

CULTURE EXPERIMENTS

To investigate the calcifugous habit of *E. colonum*, pot culture experiments were undertaken. Two sets of five culture pots each were filled with non-calcareous unmanured soil. In each set, one pot was kept as an untreated control (Pot No. 1) and to other pots, varying doses of lime were added and the following levels of exchangeable calcium were obtained at the end of the culture experiments (after about one month);

Pot No. 1 Soil with 4·8 m.e. % of exchangeable calcium

„ 2 „ 12·0 „ „ „

„ 3 „ 28·6 „ „ „

„ 4 „ 30·2 „ „ „

The detailed analysis data of culture pot soils are set in Table VI.

TABLE VI

Analysis of soils in culture pots

Soil analysis	Pot numbers				
	1	2	3	4	5
pH	7·2	7·4	8·2	8·2	8·6
Exchangeable calcium (m.e. %)	4·8	12·0	15·0	28·6	30·2
Carbonate content (%) ..	0·019	0·620	1·340	2·600	3·800

In each pot of one set, 100 seeds of the tall form and in those of the other set the seeds of the short form were put for germination. The pots were moderately watered twice a day. After noting the percentage germination in different culture pots, two identical seedlings were allowed to grow in each pot to see their performance. The results are shown in Table VII.

Maximum germination is obtained in the non-calcareous soil of the controls and with increase in dosage of lime, the germination percentage decreases in both the ecotypes. Performance of the plant was studied after about one month growth and from the data given in Table VI, it is seen that the growth is good only in pots 1 and 2, for both the ecoytpes. In calcareous soils the growth is miserably poor, the plants exhibiting stunted growth. In these soils, the plants have only a couple of leaves which usually turn chlorotic and die away soon (lime chlorosis).

A comparison of Tables VI and VII shows that both the ecotypes thrive well in soils with 4·8 and 12·0 m.e.% of exchangeable calcium and beyond this range, the plants exhibit stunted growth.

TABLE VII

Performance of E. colonum *in soils with different levels of calcium*

Culture pots	Germination %		Performance of the plant				No. of panicles	
	Tall form	Short form	Height of plant (cm.)		Dry weight of shoot (g.)		Tall form	Short form
			Tall form	Short form	Tall form	Short form		
1	78	74	33·5	24·0	1·290	0·800	2	5
2	72	70	29·0	19·0	0·988	0·350	4	3
3	66	68	9·5	6·7	0·025	0·022	1	Nil
4	52	60	7·0	6·0	0·025	0·012	Nil	Nil
5	26	28	4·5	3·8	0·020	0·011	Nil	Nil

ROOT SYSTEM

E. colonum has a fibrous root system. In keeping with the size of the shoot of the two ecotypes, the root growth is also markedly different as shown in Table VIII (Plate IV, Fig. 1).

TABLE VIII

Root system of E. colonum

Observations	Ecotype	
	Tall form	Short form
Average depth of root system (cm.) ..	21·0	9·30
Average fresh weight of shoot (g.) ..	39·54	1·43
Average fresh weight of root (g.)	14·35	0·26
Average dry weight of shoot (g.)	4·94	0·21
Average dry weight of root (g.)	2·43	0·05
Root/shoot ratio (fresh weight basis) ..	0·363	0·182
Root/shoot ratio (dry weight basis) ..	0·492	0·238

The root system of the tall form is most profusely developed than that of the short form. The lower value for the root/shoot ratio of the short form appears to be due to frequent arrest of growth due to scraping, grazing, etc.

BIOTIC FACTORS

The plants are frequently subjected to grazing and scraping, especially the short form.

The following fungal parasites are reported from India on this species (Butler and Bisby, 1931):

1. *Phyllachora graminis*—on the leaves.
2. *Ustilago trichophora*—in the ovaries.

PHENOLOGY

In nature, seeds germinate just after the first few showers, in the beginning of July. Flowering and fruiting start by the middle of August and continue till the end of September. By the middle of October almost all the plants complete their life-cycle and die away after shedding the grains.

GERMINATION OF SEEDS

The seeds of *E. colonum* have a dormancy period of about two months. Seeds collected on 1st September 1957 were used for germination studies. Seeds were put for germination, in petri-dishes, in between moist filter-papers.

It is seen that the percentage germination increases with dry storage and a maximum of 96 per cent. is obtained in the next rainy season (seeds collected in September 1957 giving maximum percentage germination in June 1958). In the following winter the percentage tends to fall (Table IX).

It is observed that the seeds collected in September can germinate in the following winter, but in nature the plants are not found growing in this season. This has been accounted firstly as due to the very poor germination of the seeds in drier soils and secondly as due to the inability of the seedlings to withstand the low temperature of winter. It may be mentioned here that the seedlings raised up in culture pots in January 1957 turned chlorotic and died away.

To investigate whether the confinement of the tall form to water-logged soils and the short form to drier localities is related to germination behaviour of these two forms, experiments were conducted with soils maintained at different moisture levels. Two sets of four petri-dishes each filled with weighed

TABLE IX

Germination of seeds of E. colonum *after different periods of storage*

Date	Percentage germination
5–9–1957	0
5–10–1957	0
5–11–1957	18
2– 1–1958	38
23– 6–1958	96
9–12–1958	78

amount of soil was taken. The soil was saturated with water and then allowed to dry, frequently weighing the dishes. Thus, the moisture in the soils was brought to the required level in each dish. The moisture lost by evaporation was added daily. Thus, the following moisture levels of 10, 20, 30 and 40% were maintained in the two sets.

The tall form gives germination of 2%, 96%, 94% and 78% respectively ; and the short form gives germination of 14%, 92%, 94% and 72% respectively in soils with 10, 20, 30 and 40% of moisture. No definite correlation could be established between germination behaviour and the distribution of the two ecotypes. It is seen that percentage germination is maximum at a moisture level of 20 and 30%, for both the ecotypes. In drier soils (10%) the germination is very poor.

Germination of seeds of *E. colonum* was tried under different light conditions. Seeds were put for germination on 2nd January 1958 in between moist filter-papers, in petri-dishes which were kept in (1) diffused daylight and darkness at night by the side of a window, (2) continuous light of an electric bulb, and (3) continuous darkness inside a chamber. At the end of the experiment, on 20th January 1958, 78% germination was obtained in continuous light, 38% in diffused daylight and 6% in continuous darkness.

Germination in continuous light is maximum and very poor germination is obtained in total darkness. The higher germination percentage in con-

inuous light may partly be due to the warm atmosphere around the electric bulb and partly due to the effect of light itself.

REPRODUCTIVE CAPACITY

Salisbury (1942) defines reproductive capacity as the product of the average seed output and the fraction represented by the percentage germination. Thus, the reproductive capacity for the tall and the short forms of *E. colonum* in nature and under cultural conditions are given in Table X.

TABLE X

Reproductive capacity of E. colonum

Ecotype	Percentage germination	Seed output	Reproductive capacity
Tall form (in wild) ..	96	2835	2722
Short form (in wild) ..	96	541	519
Tall form (in cultures) ..	96	20264	19453
Short form (in cultures) ..	96	1387	1332

Table X shows that the reproductive capacity of the tall form is much greater than that of the short form. Higher reproductive capacity values as compared to wild populations are obtained under cultural conditions due to the higher seed output values for the two ecotypes.

Vegetative reproduction of *E. colonum* is not frequent. However, roots are struck wherever nodes come in contact with the soil and give rise to new shoots at these regions. These when separated can give rise to independent plants.

OSMOTIC PRESSURE OF THE PLANT SAP

The osmotic pressure of the plant sap, under different moisture levels in the soil, is set in Table XI. It is found that the osmotic pressure of the plant sap increases with a decrease in the moisture content of the soil. Moreover, the osmotic pressure values for the tall form are lower than that for the short form.

TABLE XI

Osmotic pressure of the plant sap of E. colonum

Locality	Moisture content of sub stratum (%)	Osmotic pressure (atmos.)	
I	27·6	7·574	Tall form
II	23·1	8·720	Tall form
III	10·2	12·342	Short form

DISPERSAL OF SEEDS

Ridley (1930) mentions that the seeds of *E. colonum* are distributed, by cattle eating the panicle with the foliage and passing the grains along with the fæces uninjured. This type of dispersal appears to be very important, as the cattle at Varanasi greedily graze upon this grass. He also discusses the dispersal of the seeds by ducks.

Wind also helps to carry the seeds to short distances. Man, in the process of cutting away this grass for feeding the cattle, may bring about the dispersal of the seeds to some distance.

SEEDLING MORPHOLOGY

Seeds are broadly elliptic in outline. A few days after the seeds are put for germination, whitish unicellular hairs make their appearance at one end of the seed preceding the protuberance of the radicle. These hairs arise from the coleorhiza and resemble root hairs except that they are longer. The radicle penetrates through the coleorhiza rupturing it in this process. When the radicle is a few mm. long, the plumule arises as a protuberance from the opposite end and lengthens by the elongation of the mesocotyl and gives rise to the first leaf.

DISCUSSION

The demonstration of the existence of edaphic ecotypes implies a level of ecotypic differentiation within the species besides the climatic and geographic levels. Kruckeberg (1951) has differentiated two edaphic ecotypes in *Achillea borealis* Californica (Clausen *et al.*, 1948) in relation to serpentine and non-serpentine soils. Edaphic ecotypes have been differentiated by the present

worker also in *Euphorbia hirta* Linn., *Euphorbia thymifolia* Linn., and *Setaria glauca* Beauv. (Ramakrishnan, 1959). In *Echinochloa colonum* Link. they are mostly related to the moisture conditions of the substratum.

Salisbury (1942) has recognised the possibility of a difference in seed weight between two different strains of the same species. Such a difference in seed weight has been recognised in ecotypes differentiated in *E. colonum*. This difference in seed weight was discernible even when the two populations are raised in a neutral substratum, proving thereby its stability.

Whether large seed weight has in any way contributed to the attainment of large plant size within a species, or whether it is an inevitable accompaniment of the latter is not clear. Gregor (1946) recognises that, where the seeds are of the same weight, hereditarily large-size plants tend to produce the most vigorous seedlings. Besides the morphological characteristics, the part played by the physiological set-up of the individuals should also be taken into consideration in determining as to which variate will suit a particular habitat. If to seed size are added reproductive capacity and hereditary plant size, sea plantains tend to be larger and taller (Gregor, 1946). This observation holds good in the present case also as the ecotypes having higher seed weight are hereditarily larger in size with a higher seed output.

The production of roots is proportionate to the size of the shoot and, accordingly, it has been noted that the tall form has more profusely developed root system compared to the short form in *E. colonum*.

It has been noted that the osmotic pressure of the plant sap increases with a decrease in the moisture level of the soil. Hence, it is natural that the short form has higher osmotic pressure values compared to the short form.

As mentioned earlier, *E. colonum* is a calcifuge. Calcifuges grow in calcium-poor soils and where acidity is high, it becomes really difficult to say whether the plant is really a calcifuge in the literal sense of the word (De Silva, 1934). In *E. colonum*, at least, the soils analysed are all alkaline in reaction, but the plant appears to be a calcifuge in its true sense and the reaction appears to be of no significance as regards the calcifuge habit of the species.

Table IV shows that, in nature, the short form grows occasionally in calcium-rich soil. But, the cultural studies indicate that, beyond 12·0 m.e. per cent. of exchangeable calcium in the soil, the seedlings die away due to chlorosis of leaves. Salisbury (1920) explains the occasional occurrence of calcifuge species in calcareous soils, as a consequence of the leaching of the surface layers, so that the youth of the calcifuge plant, which is the critical

period, is completed whilst rooted in the non-calcareous substratum. This observation of Salisbury (1920) can account for the occasional presence of the short form of *E. colonum* in calcareous soils.

The chlorosis of the leaves of calcifuges have been noted by various workers, *e.g.*, McVean (1953) in *Alnus glutinosa*, Tansley (1917) in *Galium saxatile* and also by the present worker (Ramakrishnan, 1959), in the case of the obligate calcifuges in *Euphorbia thymifolia*.

In nature, *E. colonum* gives a higher seed output value in calcium-poor soils than in comparatively calcium-rich soils. This, along with other observations like ' lime chlorosis ' of the leaves, poorer germination of the seeds and performance of the plants in calcareous soils, necessitates its grouping under calcifuges.

Dormancy induced in seeds by unfavourable conditions of germination has been called ' secondary dormancy ' (Crocker, 1916). Crocker and Barton (1953) recognise that many of these conditions may be present in the soil, thus accounting for the presence of viable non-germinated seeds. As mentioned earlier, *E. colonum* has only a dormancy period of about two months and this is broken after dry storage of the fresh seeds for about two months. Though the percentage germination is poor in seeds stored for two months, still at least a few of them are capable of germination. However, no seedlings are to be found in nature during winter months and in summer. The seeds give very poor germination in drier soils and in nature ' secondary dormancy ' may be induced in the seeds due to the unfavourable germination conditions prevailing in the soil. This is evident from the fact that even if rains occur in winter, or at the end of winter, no seedlings come up in nature. Even if the seeds are made to germinate, under cultural conditions, the young seedlings turn chlorotic and die away very soon.

It has been noted in the case of *E. colonum* that during the germination of seeds, there is a profuse growth of long hairs from the coleorhizal sheath. Nishimura (1922) working with *Phleum pratense* and *Poa pratensis* has attributed protective and absorptive functions to these hairs. Haberlandt (1914) working with *Briza minor*, Nishimura (1922) with *Phleum pratense* and *Poa pratensis* and Schwartz (as quoted by Troughton, 1959) with *Panicum miliaceum* and *Setaria italica* have noted that hairs produced from coleorhiza are analogous to root hairs. In the case of *E. colonum*, and *Setaria glauca* Beauv., where also a similar phenomenon has been observed (Ramakrishnan, 1959), they resemble root hairs except that they are longer.

SUMMARY

1. This paper deals with the ecology of *Echinochloa colonum* Link.

2. Two ecotypes have been distinguished in this plant (i) the tall form, growing in very moist to water-logged soils and (ii) the short form, growing in drier localities. Differences have been recognised in the vegetative growth, seed output, reproductive capacity, average weight of the seeds, root system, osmotic pressure values of the plant sap, etc.

3. The plant is typically a calcifuge. The performance of the two ecotypes is better in non-calcareous soils than in calcareous soils. In cultures, the percentage germination decreases in calcareous soils and the seedlings exhibit 'lime chlorosis'. The occasional presence of the short form in calcareous soils is accounted as due to the passage of the seedling stage in leached non-calcareous substratum.

4. Grazing by cattle and scraping by man have been recognised as the most important of the biotic factors besides the fungal parasites mentioned.

5. The germination of the seeds after different period of storage, under different light and moisture conditions, have been studied.

6. The osmotic pressure values have been found to fluctuate with moisture content of the substratum.

7. The dispersal of the seeds and seedling morphology have been recorded.

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EXPLANATION OF PLATES

PLATE III

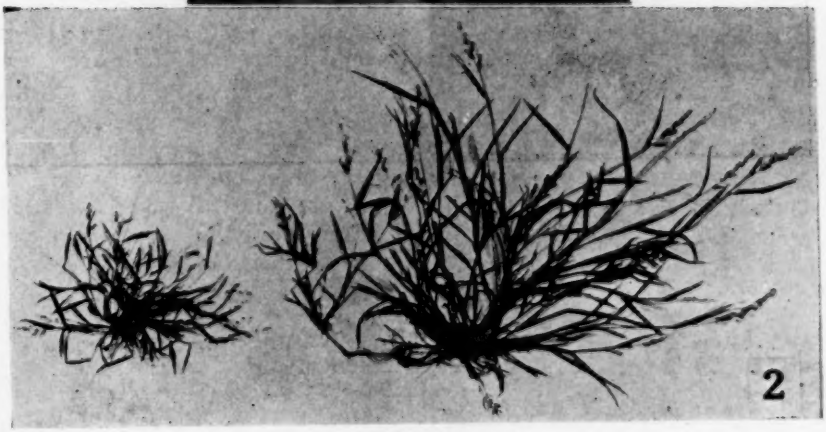
FIG. 1. Short form (left) and tall-form (right) collected from wild population. ×1/6 approx.

FIG. 2. Short form (left) and tall form (right) raised up in a neutral substratum by transplantation. × 1/7 approx.

PLATE IV

FIG. 1. Extent of development of the root system of the tall form (left) and the short-form (right) in nature. × 1/3 approx.

FIG. 2. Short form (left) and tall form (right) raised up in neutral substratum, from seeds. × 1/12 approx.



FIGS. 1–2

FIGS. 1–2

OBSERVATIONS ON THE EARLY DEVELOPMENT OF *STOMOPNEUSTES VARIOLARIS* AGASSIZ[1]

By H. P. C. Shetty[2]

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(Communicated by Dr. B. S. Bhimachar, F.A.SC.)

Introduction

The pioneer work of Johannes Muller (1853) on the external development of Echinoid larvæ has been followed by more intensive studies by many workers, notably Agassiz (1864, 1872–74), Mortensen (1903, 1913, 1921, 1931, 1937, 1938, 1948, etc.), MacBride (1903) and Onoda (1936, 1938). These investigations have brought to light the importance of larval structure in the classification of Echinoids.

By a comparison of the larvæ described by Muller (*op. cit.*) and others, Agassiz (1872–74) came to the conclusion that there are " quite marked types of embryos corresponding more or less to the several subordinal and family divisions of the Echinii ". Mortensen (1921), who worked on a number of forms from both the tropical and temperate regions of the world, considered that there is a distinct interrelationship between the larval type and the group to which the adults belong, each larval type having its own peculiar characteristics, which remain uniform within the particular family or suborder. Onoda's (1936, 1938) description of the larvæ of a number of Japanese Echinoids has confirmed the view of Mortensen.

Of the Indian forms, only *Salmacis bicolor* and *Temnopleurus toreumaticus* have so far been studied by Aiyar (1935) and Anantaraman (1939) respectively, and hence it was felt that a study of the larval development of the monotypic tropical family Stomopneustidæ, represented by *Stomopneustes variolaris* Agassiz and a comparison of its larval structure with those of closely allied forms would be of interest, especially because of its importance in classification, as envisaged by Mortensen (1921).

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[1] Taken from the Thesis submitted in partial fulfilment for the M.Sc. Degree of the University of Madras, 1956.

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MATERIAL AND METHODS

The materials required for the investigation undertaken were collected from the rocky intertidal region of the Madras coast and the various stages of development were obtained by artificial fertilization. The specimens were cut open and bits of gonads were removed to glass vessels containing fresh filtered sea-water. The ovary was cut into small bits and by drawing them in and out of a pipette, the eggs were liberated into a number of finger bowls, containing filtered sea-water. The testis was also cut into bits and put into a bowl of filtered sea-water, when the spermatic fluid was liberated into the water.

The eggs and sperms were also obtained by allowing the ripe males and females to spawn in glass troughs containing fresh sea-water. The eggs and sperms collected in masses over the genital plates, and could easily be removed to bowls containing fresh filtered sea-water.

A drop or two of the spermatic fluid were added to each of the finger bowls, containing a few eggs. The contents were then stirred to facilitate the mixing of eggs and sperms. Care was taken not to put too many eggs and sperms into the bowls. Some of the bowls were kept in trays containing about three inches of water, in order to keep down the temperature.

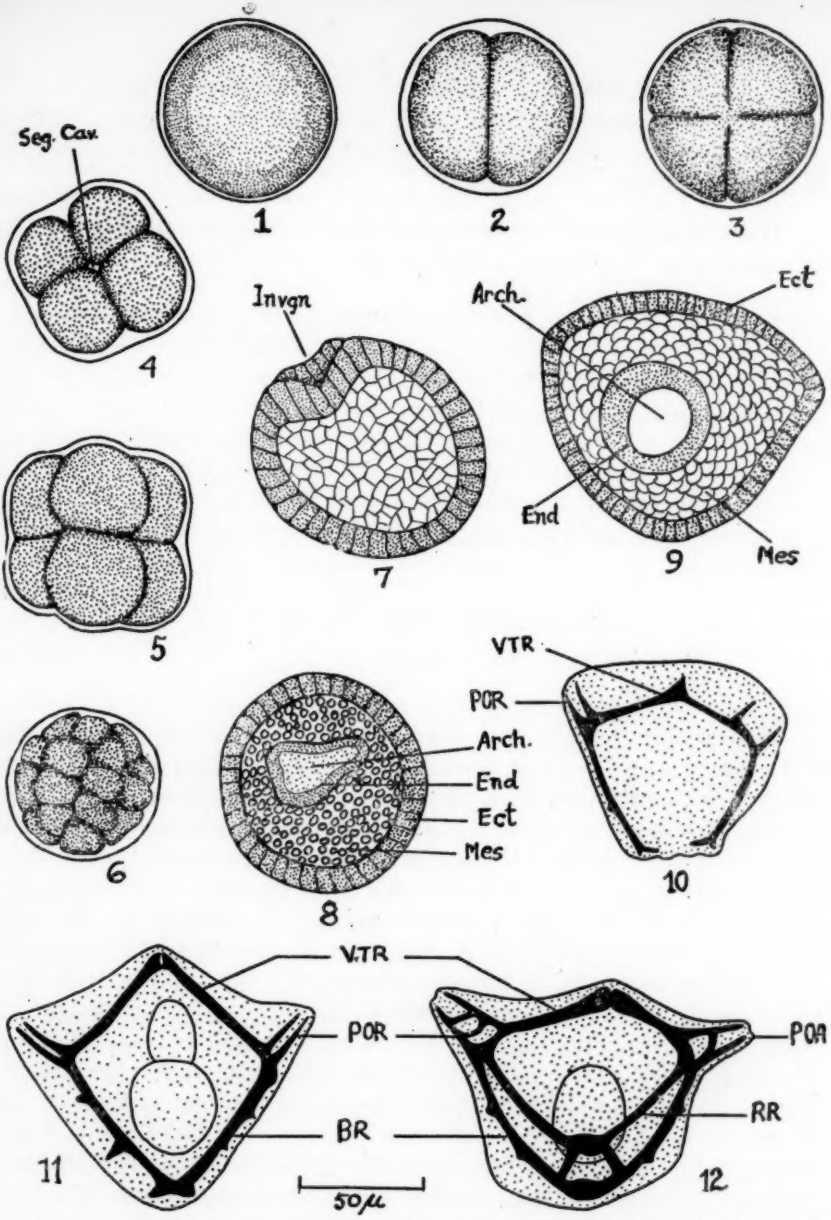
Every day the embryos were picked out with a pipette and transferred to fresh bowls containing filtered sea-water. The frequent change of water, in addition to providing fresh supplies of oxygen and food, also helped in reducing the number of ciliates which prey on the embryos. The larvæ were fed on the diatom *Nitzschia* spp.

The various stages were studied mostly in the living condition. They were also studied later on from stained wholemounts.

OBSERVATIONS

The freshly removed ova are of irregular shape, beacuse of close packing inside the ovary. When left for some time in fresh filtered sea-water, they become perfectly spherical. The ova are small, varying mostly from 64 μ to 68 μ in diameter, some being even as small as only 54 μ across. They are almost opaque, with the cytoplasm more granular and slightly yellowish towards the periphery (Fig. 1).

On mixing the eggs and sperms in sea-water, each egg is seen surrounded by numerous actively swimming sperms, of which only one succeeds in entering the egg and fertilization is thereby effected. Following this, starting at



FIGS. 1–12. Fig. 1. An egg at the time of fertilization. Fig. 2. Two-celled stage. Fig. 3. An egg seen from above; the second cleavage is in progress. Fig. 4. Four-celled stage; second cleavage completed. Fig. 5. Eight-celled stage; viewed along the equitorial plane. Fig. 6. Thirty-two-celled stage. Fig. 7. Late Blastula (semi-diagrammatic), about 20 hours old; Gastrulation just setting in. Fig. 8. Gastrula, 24 hours old. Fig. 9. Late Gastrula—42 hours old. Fig. 10. Beginning of the Pluteus form, 42 hours old. The ventral transverse rods have joined together. Fig. 11. Early Pluteus stage, 43 hours old. The body rods have fused with each other. Fig. 12. Early Pluteus, 44 hours old. The recurrent rods have appeared.

the point of penetration of the sperm, a fertilization membrane, which is quite thin to start with, separates out and this seems to prevent the entry of further sperms.

The following is the chronological order of events taking place after fertilization up to the first pluteus stage:—

1st division	2 cells	..	1 hour
2nd „	4 „	..	1 hour 10 minutes
3rd „	8 „	..	3 hours
4th „	16 „	..	3½ hours
5th „	32 „	..	5 hours
Morula ..	..	..	6 hours
Beginning of gastrulation		..	About 20 hours
End of gastrulation		..	24 hours
Beginning of the pluteus form			44 hours
Completion of the I Pluteus Stage ..	..	..	90 hours

The cleavage which starts within an hour is total and regular. The first cleavage which is vertical, starts as a cleft in about 55 minutes after insemination. Within the next three minutes a complete vertical furrow is formed extending between the two poles and thus in about an hour, the two-celled stage is reached (Fig. 2). Now the membrane becomes more prominent and appears gelatinous, and the egg at this stage measures about 69 μ. The next division, which is also meridional, but at right angles to the first, takes place in another ten minutes, *i.e.*, in 1 hour 10 minutes after insemination. The resulting four cells are pressed against each other all along their sides, but in the centre is left a small space or cavity, which is the beginning of the blasto-cœlic cavity (Figs. 3 and 4). The diameter at this stage is about 71·4 μ. The third division, which takes place in about three hours, is equatorial and divides the egg into eight cells (Fig. 5). In another half-an-hour the egg divides for the fourth time, resulting in 16 cells. The fifth division is also regular and is completed in five hours after fertilization (Fig. 6). Divisions continue to be regular and a morula is formed in about 6 hours. This leads on to the blastula stage.

The blastula attains a diameter of about 85–98 μ. It is less opaque than the preceding stages and is still covered by the membrane. It rotates about vigorously in the water. The cells appear columnar now because of the pres-

sure on the sides by the neighbouring cells and present a polygonal outline on the outer surface. The cells at one end have become slightly longer and more columnar than the rest, and this end marks the vegetal pole. The blastula gradually assumes an oval form from the original spherical form, and measures about $98 \times 86\ \mu$.

Gastrulation sets in about 20 hours after fertilization and results in the invagination of the columnar cells at the vegetal pole into the blastocœl (Fig. 7). This end is the blastoporal end. The invagination deepens into a narrow tube, the archenteron, and extends to very nearly the other pole. The process of gastrulation is completed in about 24 hours (Fig. 8). MacBride (1903) has described the process of gastrulation in *Echinus esculentus*. He found in the interior of the blastula a fluid proteid, which may be compared to the yolk in larger eggs. He suggests that as this proteid solution thins out, the inside pressure gets reduced and so the growing part of the wall of the blastula at the blastoporal end naturally bends inwards to form the archenteron. The same process is probably involved in the gastrulation of *Stomopneustes variolaris*.

Soon after gastrulation, cells from the outer wall escape into the interior and come to occupy the space between the outer layer and the archenteron. These are the mesemchyme cells, which are destined to give rise to the connective and muscular tissues (Fig. 9). Early in the second day, a tri-radiate spicule is formed on each side of the archenteron, arising from the mesenchyme cells. The appearance of these spicules marks the beginning of the bilateral symmetry of the ' Echinopluteus ' larva. Of the three rays of each spicule, one is directed anteriorly, another towards the middle and the third directed posteriorly and these represent the rudiments of the ' postoral ', the ' ventral transverse ' and the ' body ' rods respectively. Towards the latter part of the second day the embryo tends to become conical, with a broad anterior part and a narrow posterior part. The two ventral transverse rods meet and fuse in the mid-line, and the other rods also grow longer, specially the body rod. The gastrula appears transparent at this stage (Fig. 10).

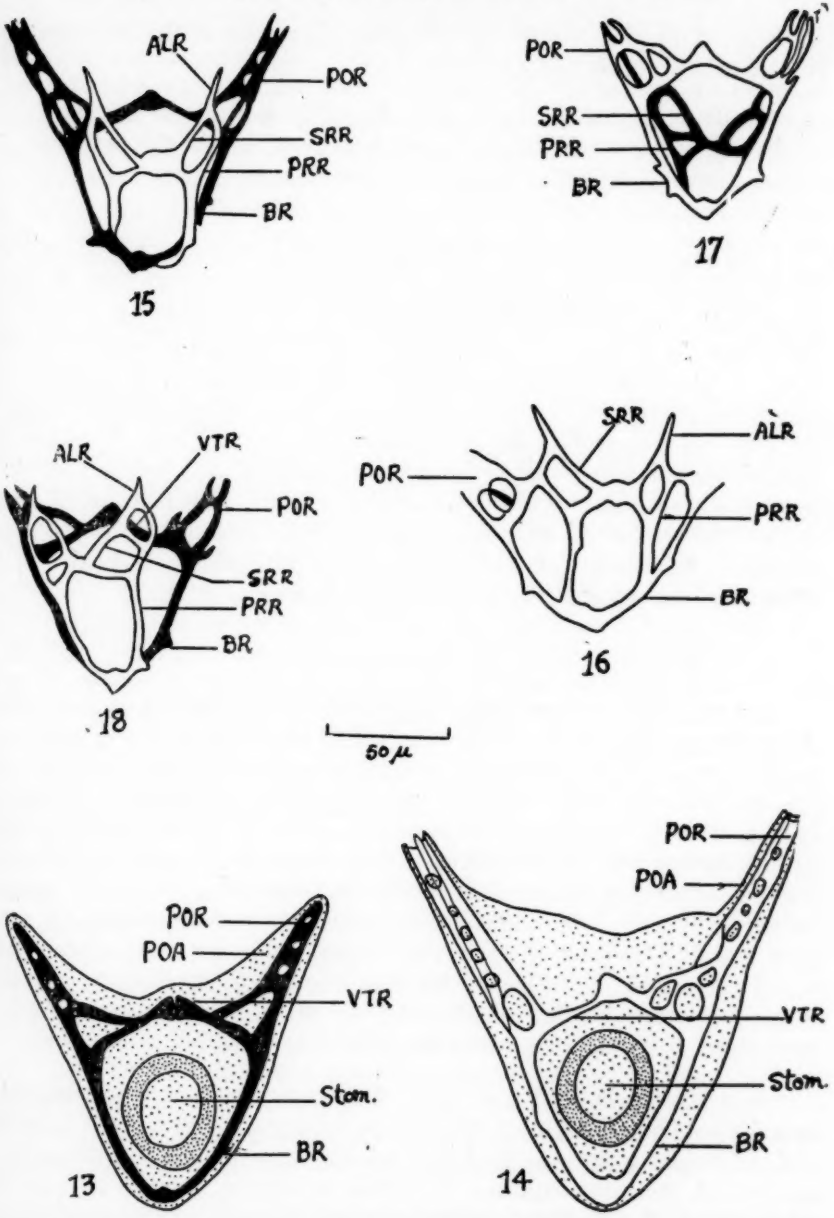
In the meanwhile, development of the alimentary tract has been taking place. The archenteron remains narrower towards the blastoporal end and the anterior end is slightly dilated. The blastopore persists as the anal opening. An ectodermal stomodæal depression takes place anteriorly and this later on fuses with the anterior ' stomach ' region of the archenteron. On the third day, the œsophagus, which is partly ectodermal and partly endodermal, shows signs of life by its pulsations and feeding starts at this stage

through the mouth-opening. The original archenteron becomes gradually differentiated into the œsophagus, the stomach and the intestine.

Larval form.—At the age of 44 hours there appears the first indication of the pluteus form, in the formation of slight tubular outpushings at the two antero-lateral corners. These are the rudiments of the postoral arms, which are the first pair of arms to be formed (Fig. 12). The rudiments gradually become more and more prominent and by about 68 hours a pair of short stumpy postoral arms are developed. These become still more elongated and reach their full length by about 90 hours. They are supported by the arm skeleton and the cilia originally found all over the gastrula, are now confined to the edge of the arms.

Larval skeleton.—By the end of the fourth day, the larval skeleton of the early pluteus stage is fully formed (Figs. 15–18). It is a framework of calcareous rods, supporting the body and the arms of the larva. It is noteworthy that the skeleton of this species forms a compound basket structure.

In the three-day-old larva, the skeleton consists of (i) a 'postoral' rod on either side, fenestrated throughout its length, forming the supporting framework of the postoral arms, which are the first pair of arms to develop ; (ii) a pair of 'ventral transverse' rods, which branch off from the two postoral rods and grow towards the middle where they meet and fuse together to form a single structure ; (iii) a pair of 'body' rods, which proceed along the lateral margins of the larva towards the posterior middle line, where they fuse with each other ; they bear a few variously directed lateral spines and anteriorly these body rods are continuous with the postoral rods ; and (iv) a dorsal branch that proceeds from the junction of the body rod with the postoral rod and which divides into the 'antero-lateral' and the 'recurrent' rods. The antero-laterals of the two sides are separate from each other and are directed anteriorly. They form the skeletal framework for the second pair of arms, namely, the antero-lateral arms, the rudiments of which are developed in a few larvæ even at the age of 72 hours. The recurrent rods form a complicated structure and contribute to the compound basket structure of the skeletal framework. There are two recurrent rods on either side, the 'primary' and the 'secondary'. The primary and the secondary recurrent rods of the two sides meet in the mid-line by transverse processes, which dilate at the point of fusion. Beneath this, the primary recurrent rod alone proceeds backwards and joins the body rod. Anteriorly the primary and the secondary recurrent rods join each other and then become continuous with the antero-lateral rod at one end, and with the body rod at the other. Thus two large meshes are formed on either side of the larva. Sometimes, as in *Echinometra*



FIGS. 13–18. Fig. 13. Pluteus, 75 hours old. Fig. 14. Pluteus, 90 hours old. Fig. 15. Larval skeleton of the three-day-old larva; dorsal view. Fig. 16. Dorsal view of the larval skeleton of three-day-old larva, showing the connection between the various skeletal rods. Fig. 17. Larval skeleton of the three-day-old larva; ventral view, showing additional ramification of the recurrent rod, forming a third mesh on each side. Fig. 18. The same, seen from the dorsal side.

lacunter, there may also be smaller additional meshes at the lower end due to secondary ramifications.

In the course of the fourth day, the antero-lateral rods become longer and more pronounced. They are not fenestrated, unlike the postoral rods, which also become longer, with an increased number of fenestræ along their length.

The rate of development of the eggs was found to vary in different cultures, as well as in the same culture. There was also found a great disparity in the size of the embryos developing from the various eggs, whose size may vary from 54 to 68 μ. The development proceeded quite normally till the gastrula stage in almost all the cultures. After this, a few of the embyos only developed further into the first pluteus stage, while others continued to remain alive for some more days but in the same gastrula condition. Even the few that developed into plutei did not survive beyond the fourth day. The same result was obtained after every attempt in spite of the precautions taken. To a certain extent this must have been due to the pollution of water by the decaying of dead larvæ in the culture, since such pollution is known to be detrimental to the living larvæ (Wilson and Armstrong, 1954).

DISCUSSION

It has now been largely recognised that the larval structure of the Echinoderms throws light on the affinity of adult forms and as such, it is of classificatory value. On this basis, one would expect to find similar types of larvæ for closely related adult forms. If it is not so, then according to Mortensen (1921), the original classification would require to be reorientated in the light of the new findings. Mortensen recognised several larval orders and families and has adjusted the classification of adults in accordance with his findings on larval structure. Among the larval types he has established are the Spatangoid larval order (with some larval families within its fold), Clypeastroid larval family, Diadematid type, Arbaciid type and under the suborder Camarodonta four distinct types, characteristic of the families Echinidæ, Toxopneustidæ, Echinometridæ, and Temnopleuridæ.

Both Mortensen (1921) and Clark (1917) have recognised Stomopneustidæ as a distinct family which has been grouped along with the families Salenidæ and Arbaciidæ under the suborder, Stirodonta, and order Diadematoida. One would, therefore, expect to find close similarities in larval structure in the members of these three families. Little is known of the development of Salenidæ, which are essentially deep-sea forms. But the family Arbaciidæ

has been widely studied. There is a distinct Arbaciid larval type, characterised in its first stage by the skeleton forming a simple basket structure, the recurrent rod being single, and in its second stage by the existence of a posterior transverse rod, which ends in a pair of long postero-lateral arms of the same shape as the other arms. This feature of the recurrent rod uniting with the body rod to form a basket structure is a primitive one and this is seen specially in the Cidarids, Diadematids, and Arbaciids. A further development of the basket structure is seen in the Echinometridæ, where the recurrent rod has become double, and in Clypeastroids, where the posterior portion of the basket has become a fenestrated plate. The basket structure has been given up only in Temnopleuridæ and Echinidæ and also in the genus *Lytechinus* of Toxopneustidæ. Further, the fenestrated rods represent a primary structure, characteristic of the primitive forms. Both the basket structure of the skeleton and the fenestrated nature of the skeletal rods of the arms are seen in *Stomopneustes variolaris*, and as such it is evident that Stomopneustidæ constitute a primitive group of Echinoids.

The larva of *Stomopneustes variolaris* resembles the Arbaciid larval type (*e.g.*, *Arbacia punctulata*) in forming a basket structure in its first stage and also in the fenestrated nature of the postoral arms. But the basket structure in the former is a compound one, with two recurrent rods—primary and secondary—on either side. Then again in the majority of Arbaciids, as for example, *Arbacia lixula* and *A. stellata*, the postoral rods are fenestrated only towards their apex. However, in *Arbacia punctulata* the postoral rods are fenestrated right from the base as in *Stomopneustes variolaris*. Whether resemblance occurs in the later stages too is not known, as there is no record of the later larval stages of Stomopneustidæ.

On the other hand, the early larval stage of *Stomopneustes* shows a marked similarity to the larval structure of the members of the family Echinometridæ, which are grouped under a separate suborder Camarodonta. In both these groups, the recurrent rod is double, forming a compound basket structure with the body rod. Such a structure shows two large meshes on each side, and Mortensen (1921) has reported the formation of smaller additional meshes at the lower end due to secondary ramifications in *Echinometra lacunter*. This also has been observed in *Stomopneustes variolaris*. Further, in both the groups the body rods and the ventral transverse rods join their fellows in the mid-line, at which point they are slightly dilated. The postoral rods are fenestrated throughout their length in the two groups.

This type of compound basket structure is seen also in *Echinostrephus moralis* (Onoda, 1936), which is included in the family Strongylocentrotidæ

by Clark (*op. cit.*). But Mortensen grouped it in the family Echinometridæ which show the same characteristics as pointed out above. It is difficult to draw conclusions at this stage regarding the systematic position of *Stomopneustes variolaris* in the absence of knowledge of its later development. However, its early development indicates its marked affinity to the Echinometridæ and if this can further be substantiated, it might largely justify Lankester's (1900) classification, wherein he included *Stomopneustes* in the family Echinometridæ. But Mortensen (1921) and Clark (1917) have grouped them under two different suborders on the basis of adult features. Since larval structure is indicative of affinities of the adult forms, it may be stated, that the Stomopneustidæ and Echinometridæ are, at least, closely related groups, nearer to each other than recognised at present. Further investigations would be required to elucidate the exact taxonomic position of this group.

The rate of growth in animals is known to be governed by a number of physical, chemical and biological factors in the environment, and it is known from the work of Mortensen (1921), Aiyar (1935), Paul (1942) and others that the rate of growth is generally more rapid in the tropical regions, because of the higher temperatures there accelerating the metabolic activities. As for example, the work of Anantaraman (1939), Mortensen (1921) and Onoda (1936) has shown that the rate of development of *Temnopleurus toreumaticus* in tropical Madras is about double that of the same species in sub-tropical Japan. But temperature alone does not account for all the observed differences in the rate of development, as could be seen in Tennent's (1929) 55-hour old larva of *Salmacis virgulata* being very similar to Aiyar's (1935) 10-day-old larva of *S. bicolor* in spite of similar temperature conditions at Torres Straits and Madras respectively.

Stomopneustidæ being a monotypic family restricted to the tropical waters of India, Australia and Africa, and in view of its uncertain systematic position in the light of the findings discussed here, it is not possible to compare its rate of growth with other apparently related forms. However, the rate of growth of the early larva as observed here might be of use for comparison with data obtained by future work on the same or closely related species. Incidentally its rate of growth was found to be decidedly slower than that of two other Madras Echinoids so far studied, *viz.*, *Salmacis bicolor* (Aiyar, 1935) and *Temnopleurus toreumaticus* (Anantaraman, 1939), while it showed remarkable similarity to that of some other tropical forms like *Echinometra oblonga* and *Tripneustes esculentus* (Mortensen, 1921), both of which show the basket type of larval skeleton.

Summary

1. The early development of *Stomopneustes variolaris* Agassiz has been traced from artificially fertilized eggs up to the end of the fourth day.

2. The details of segmentation and gastrulation are given. The blastocœl is traceable even from the four-celled stage.

3. The larval skeleton resembles the Echinometrid type, forming a compound basket structure.

4. Affinities of the form based on larval structure are discussed. The form shows close resemblance to Echinometridæ, which are presently grouped under a different suborder.

Acknowledgement

I have great pleasure in acknowledging my indebtedness to Dr. G. Krishnan, Reader, University Zoological Research Laboratory, Madras, where this work was carried out, for his constant guidance and helpful criticisms in the course of this investigation. I am also thankful to Dr. C. P. Gnanamuthu, Director, and to Dr. S. Krishnaswamy, Senior Lecturer, of the above Laboratory for their kind help. I am further indebted to Dr. T. V. R. Pillay for his helpful suggestions in the preparation of this paper.

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KEY TO LETTERING

ALR, Antero-lateral rod.

Arch, Archenteron.

BR, Body rod.

Ect, Ectoderm.

End, Endoderm.

Invgn, Invagination

Mes, Mesenchyme.

POA, Postoral arm.

POR, Postoral rod.

PRR, Primary recurrent rod.

RR, Recurrent rod.

Seg. Cav., Segmentation cavity.

SRR, Secondary recurrent rod.

Stom. Stomach.

VTR, Ventral transverse rod.

STUDIES IN PECTIC ENZYMES OF PARASITIC FUNGI

III. Secretion of Pectic Enzymes by *Penicillium expansum* Link.

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(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

INTRODUCTION

Penicillium expansum Link. has been reported to cause soft rot in different fruits from several parts of India (Singh, 1941; Singh and Grewal, 1953; Gupta and Pandey, 1959 *a*). The fungus has also been reported to secrete protopectinase enzyme on the fruits which are attacked by it and on several natural media (Gupta, 1960 *a* and 1960 *b*). Earlier, *P. ehrlichii* (Ehrlich, 1932) and *P. notatum* (Lulla and Johar, 1953) were found to secrete polygalacturonase (PG) enzyme. *P. chrysogenum* has been reported to secrete protopectinase, polygalacturonase and pectin-esterase (PE) enzymes (Phaff, 1947) while in the case of *P. citrinum* (Roboz and Kertesz, 1948) PE was found to be absent (definitions of the enzymes are given by Kertesz, 1951).

In the present work the author reports the secretion of different pectic enzymes in culture media by *Penicillium expansum*.

MATERIALS AND METHODS

The strain of *Penicillium expansum* used in this work was locally isolated from infected apples and the culture was maintained on potato-dextrose agar slopes at 24–25° C.

For the preparation of pectic enzymes the fungus was grown on 15 ml. of culture medium taken in each flat bottle of 12 fluid oz. capacity. The details of sterilization of media, inoculation of the fungus and incubation of the cultures are given in the earlier communication (Gupta and Pandey, 1959 *b*). The following media were prepared:

(*a*) *Lucerne medium*.—Various percentages of powdered lucerne seeds (*Medicago sativa*) were taken in distilled water in a beaker and kept in boiling water-bath for an hour, stirring several times. The decoction was then filtered through fine muslin and the filtrate was used as culture medium.

(*b*) *Pectin asparagin medium.*—5 g. dextrose, 10 g. pection, 4 g. asparagin, 0·5 g. magnesium sulphate and 1 g. potassium dihydrogen phosphate in 1 litre of distilled water.

(*c*) *Modified Czapek's medium.*—3 g. sodium nitrate, 0·5 g. potassium chloride, 20 g. sucrose, 10 g. pectin, 0·5 g. magnesium suphate, 1 g. potassium dihydrogen phosphate and 0·01 g. ferrous sulphate in 1 litre of distilled water.

(*d*) *Dextrose pectin medium.*—25 g. pectin, 20 g. ammonium nitrate, 2 g. dextrose, 0·02 g. magnesium sulphate and 10 g. of potassium dihydrogen phosphate in 1 litre of distilled water.

The activity of protopectinase enzyme in a solution was tested by the potato disc method of Brown (1915) and has been expressed in this work as the time taken to macerate (M.T.) 3 potato discs of 11 mm. diameter and 0·5 mm. thickness when kept in 5 ml. solution of the enzyme preparation.

The polygalacturonase (PG) enzyme was assayed by following the loss of viscosity of sodium polypectate solution. For each test 5 ml. of 1·0% sodium polypectate solution and 2 ml. of enzyme preparation were separately adjusted to pH 6·2 by using 0·1 N NaOH or 0·1 N HCl and brought to 30° C. temperature in a water-bath. The two preparations were then mixed, stirred and 5 ml. of it transferred to an Ostwald viscometer in the water-bath at 30°C. The first viscosity determination was made one minute after adding the enzyme and then at intervals over a period of 20 minutes.

The activity of pectin-esterase enzyme was determined by estimating free carboxyl groups liberated in pectin solution by the action of this enzyme. For each determination 5 ml. of 1% pectin solution (pectin with approximately 10% methoxyl group) was taken in 1 oz. stoppered glass bottle and 0·1 N NaOH was added to bring the pH to 6·2. Two ml. of the enzyme preparation and 2 drops of methyl red indicator were added to the pectin solution and kept at 25° C. PE activity caused a fall in the pH and was indicated by a change in the colour of the solution. This change in colour was readjusted from time to time by adding 0·05 N NaOH.

All the pH measurements were determined by using Cambridge pH Indicator.

EXPERIMENTAL

(*i*) *Protopectinase enzyme.*—Liquid media of various concentrations were prepared by taking 10%, 5·0%, 2·5%, 1·0% and 0·5% of powdered lucerne seeds in distilled water. The cultures were incubated at 25° C. for 3 and 5 days. The results are shown in Table I.

TABLE I

Secretion of Protopectinase Enzyme on Different Concentrations of Lucerne Medium

Concentration of the medium	3 days incubation		5 days incubation	
	M.T.	Dry weight of mycelium in mg.	M.T.	Dry weight of mycelium in mg.
10%	35–40 minutes	78	60–65 minutes	65
5%	35–40 minutes	45	60–65 minutes	42
2·5%	35–40 minutes	30	60–65 minutes	20
1%	60–80 minutes	20	80–90 minutes	9
0·5%	22–24 hours	4	11–12 hours	6

M.T. = Maceration time for standard potato discs.

Decoctions of 2·5–10% lucerne were found to be equally good for the secretion of protopectinase enzyme though the growth of the fungus declined with the decrease in the concentration of the medium.

Cultures on 2·5% lucerne decoction were kept at different incubation temperatures. It was found (Table II) that incubation temperature of

TABLE II

Effect of Incubation Temperature on the Secretion of Protopectinase

Incubation temperature in °C.	Initial pH	3 days incubation			5 days incubation		
		Final pH	M.T. in minutes	Dry weight in mg.	Final pH	M.T. in minutes	Dry weight in mg.
7·5	6·5		No growth			No growth	
15	6·5	6·8	40–45	38	6·9	25–30	50
20	6·5	6·9	25–30	55	7·2	45–50	45
25	6·5	7·0	40–45	41	8·4	90–100	34
27·5	6·5	7·1	130–140	34	8·6	180–200	28

15–20° C. is the optimum for the secretion of protopectinase enzyme. The maximum activity of the enzyme was noted at 20° C. in shorter incubation period than at 15° C.

Lucerne medium of 2·5% concentration was also used to find out the optimum incubation period when cultures were kept at 20° C. It was observed that best incubation period was of 4 days. The pH of the cultures shifted towards alkanity up to 7 days incubation period and then it became constant. Autolysis of the mycelium became more than its growth in 4 days (Fig. 1).

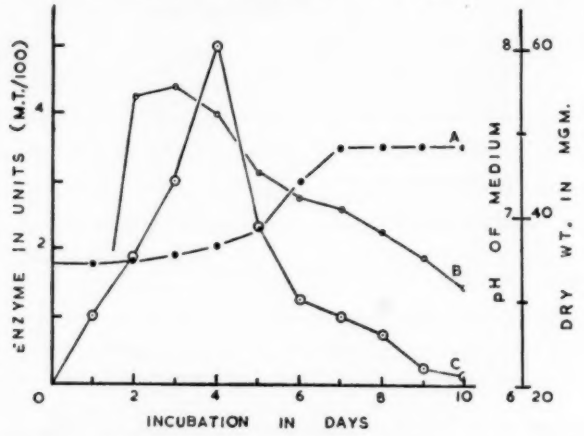

FIG. 1. Effect of incubation period on the protopectinase enzyme secretion (C), pH of the medium (A) and dry weight of the mycelium (B).

The secretion of protopectinase enzyme was examined on the following synthetic media:

M_1—Pectin asparagin medium.

M_2—Pectin asparagin medium + 0·1% NaCl.

M_3—Pectin asparagin medium where whole of the pectin was replaced with equal amount of glucose (by weight).

M_4—Pectin asparagin medium where whole of the asparagin was replaced with equal amount of ammonium nitrate (on nitrogen basis).

M_5—Modified Czapek's medium.

M_6—Modified Czapek's medium where whole of sodium nitrate was replaced with ammonium nitrate (on nitrogen basis).

M_7—Dextrose pectin medium.

Cultures were incubated for 4 days at 20° C.

It was observed that most active protopectinase enzyme was secreted on pectin asparagin and modified Czapek's media when ammonium nitrate was used as nitrogen source. Dextrose pectin medium did not support the secretion of the enzyme which could be due to high concentration of various salts and low pH of the medium. Enzyme secretion did not improve when sodium chloride was added to the medium though such an improvement was observed in the case of *Pythium debaryanum* (Gupta, 1956). Absence of pectin made a medium unsuitable for enzyme secretion which was otherwise not so. The results are shown in Table III.

TABLE III

Secretion of Protopectinase Enzyme on Synthetic Media

Medium	Initial pH	Final pH	M.T.	Dry weight in mg.
M_1	3·6	6·0	50–55 minutes	99
M_2	3·6	7·0	120–130 minutes	119
M_3	4·2	7·2	>24 hours	108
M_4	3·6	3·2	15–17 minutes	74
M_5	3·6	7·0	80–90 minutes	117
M_6	3·2	3·8	17–20 minutes	151
M_7	2·4	3·2	>24 hours	80

(*ii*) *Polygalacturonase enzyme* (PG).—Highly active PG was secreted on all those synthetic media on which active protopectinase enzyme was obtained (Fig. 2). PG of very low activity was secreted on medium where pectin was replaced with equal amount of glucose (M_3), pectin asparagin medium with sodium chloride (M_2) and dextrose pectin medium (M_7).

(*iii*) *Pectin-esterase enzyme* (PE).—Very low activity of PE was recorded in all the preparations made from different cultures after 4 days incubation at 20° C. The highest activity of PE was recorded on modified Czapek's medium where ammonium nitrate was used as nitrogen source (M_6). In this preparation only 0·15 ml. of 0·05 N NaOH solution was required to neutralize the carboxyl groups liberated during 24 hours reaction time.

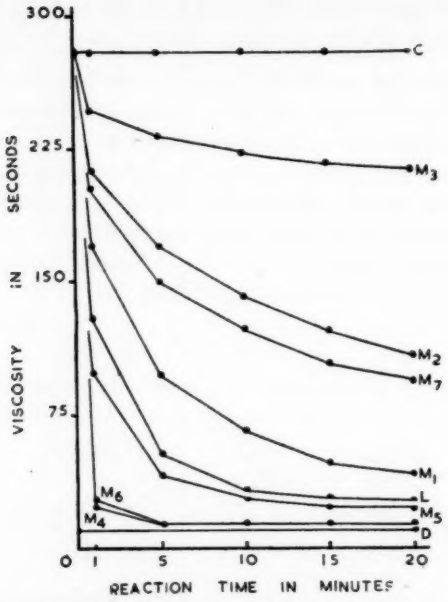

FIG. 2. Effect of polygalacturonase (PG) enzyme on the viscosity of sodium polypectate solution. C—Control with boiled enzyme; D—viscosity of the distilled water; L—enzyme secretion on 2·5% lucerne medium; M_1-M_7—enzyme secretion on different synthetic media (as mentioned in text).

SUMMARY

Highly active protopectinase and polygalacturonase (PG) enzymes were secreted by *Penicillium expansum* Link. on 2·5% decoction of lucerne, pectin asparagin and modified Czapek's media. Ammonium nitrate was found to be a better nitrogen source for the secretion of pectic enzymes than asparagin or sodium nitrate. Best secretion of protopectinase was in cultures after 4 days incubation at 20° C.

Pectin-esterase, when tested at 6·2 pH, was found to be of negligible activity in 4 days old cultures of the fungus.

Protopectinase and polygalacturonase (PG) were found to be adaptive enzymes which were not produced in absence of pectic substances.

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TRIMETHYLAMINEOXIDE CONTENT OF MARINE PRAWNS OCCURRING IN THE BACKWATERS AND IN THE SEA OFF COCHIN

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(Communicated by Dr. S. Jones, F.A.SC.)

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Trimethylamineoxide has been reported in most marine fishes and several other marine animals occurring to the extent of 1% while it is absent or present only in very small concentration in freshwater fishes.[1] This distribution suggests the probability of its having a role in the physiology, possibly in the osmoregulation, of marine fishes. The oxide is also the precursor of trimethylamine which is produced postmortem in seafish through bacterial action. The estimation of trimethylamine in fish muscle has been found useful as an index of spoilage in seafish.[2] The occurrence and distribution of trimethylamineoxide in fishes has therefore drawn the attention of physiologists and fish technologists. In this context the values of trimethylamineoxide content of marine prawns belonging to different species caught from the sea off Cochin and from the Cochin backwaters respectively determined by us recently appear to be significant.

Material and Methods

Prawns from the sea were obtained from the catches made by the trawlers operated by the Offshore Fishing Station, the Central Fisheries Technological Research Station, and the Indo-Norwegian Project, at Cochin. The hauls were taken at depths of 8–20 fathoms South-West of Cochin Port. Backwater prawns were obtained from the catches made by stake-nets and dip-nets operated in the Cochin backwaters. The trimethylamineoxide content of the prawn muscle was determined according to the procedure described previously by the authors.[3]

Results and Discussion

The TMAO content of the prawns taken from the sea and from the backwaters is given in Table I. The changes in the TMAO content of the backwater prawns during the months November 1959 to April 1960, are

TABLE I

The trimethylamineoxide content of sea prawns (caught by trawlers) and backwater prawns

Species	Sea prawns		Species	Backwater prawns	
	TMAO (mg. N/100 g. muscle)	Date		TMAO (mg. N/100 g. muscle)	Date
Peneæus carinatus	98·42	3–11–1959	*M. monoceros*	Nil	18–11–1959
Do.	85·94	Do.	*P. indicus*	Nil	20–11–1959
Metapenæus monoceros	92·06	Do.	*M. monoceros*	Nil	Do.
			P. indicus	6·2	27–11–1959
Do.	70·94	Do.	*M. monoceros*	Nil	Do.
Peneæus monodon	111·70	10–11–1959	*P. indicus*	Nil	30–11–1959
Do.	105·00	Do.	*Metapenæus dobsoni*	Nil	Do.
P. indicus	59·41	3–12–1959			
Metapenæus affinis	46·68	Do.	*P. indicus*	5·0	17–12–1959
Do.	30·91	8–12–1959	*P. monodon*	Nil	30–12–1959
Do.	44·18	Do.	*M. dobsoni*	Nil	Do.
Do.	56·94	Do.	*P. indicus*	9·45	Do.
P. indicus	32·90	15–12–1959	*P. indicus*	11·25	6– 1–1960
M. affinis	91·87	Do.	Do.	8·45	12– 1–1960
Do.	53·76	Do.	*M. dobsoni*	24·25	Do.
M. monoceros	90·84	Do.	*P. indicus*	8·44	15– 1–1960
Do.	71·78	Do.			
Do.	61·17	Do.	*M. dobsoni*	13·93	Do.
P. indicus	48·64	22–12–1959	*P. indicus*	21·70	9– 2–1960
M. affinis	96·51	Do.	Do.	19·44	Do.
Do.	118·30	Do.	Do.	26·28	Do.
P. indicus	47·54	23–12–1959	Do.	33·60	Do.
Parapenæopsis stylifera	107·10	28–12–1959	Do.	41·98	Do.
			P. indicus	25·59	19– 3–1960
		Do.		23·70	Do.
		Do.		24·48	Do.
			P. indicus	20·38	28– 4–1960
		Do.		17·30	Do.
		Do.		19·07	Do.
			P. monodon	13·02	Do.
		Do.		18·37	Do.

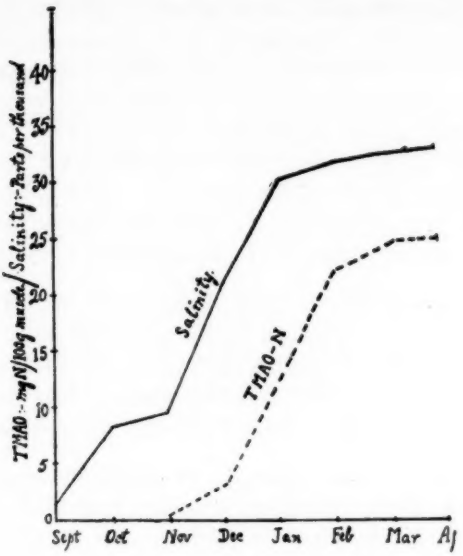

FIG. 1

shown in Fig. 1, along with the salinity curve for the Cochin backwaters during this period.*

There is considerable variation in the TMAO content of individuals of the same species in the trawler caught prawns (Table I). Individual variation has been reported in the oxide content of some species of fish, particularly cod, for which extensive data are available.[1] However the almost total absence of the oxide in the marine prawns taken from the backwaters when the salinity of the waters was very low, is noteworthy. This fact and the correlation in the increase in TMAO content with the rise in salinity (Fig. 1) suggests a physiological role for the TMAO in the backwater prawns. It may represent a detoxicated ammonia; such a detoxicating mechanism would help the prawns to resist the adverse effect of the restricted 'available' water-supply[4] during the period when the salinity of the backwaters is high.

The marine prawns breed in the sea but migrate into the backwaters early during the post-larval stage in search of a sheltered environment and a more abundant food supply.[5] Later they return to the sea where they

* The salinity curve is based on data collected by Sri. M. J. George of the Central Marine Fisheries Research Station, Ernakulam.

Note:—TMAO=trimethylamineoxide.

attain maturity. The backwater prawn fishery is therefore supported, almost exclusively by the juveniles. The size of these prawns is smaller than that of prawns caught in the sea by the trawlers. The difference in the TMAO content of the sea and backwater prawns is however too great to be explained merely by the size difference though it is known that in a given species the larger fishes contain more of the oxide. It is interesting to note in this context that the TMAO content of *Parapenæopsis stylifera* (a very small-sized prawn) is relatively very high (Table I). This species, unlike the other marine prawns, is strictly marine being never encountered in the backwaters.[5]

Baldwin[4] has expressed the view that, unlike in the elasmobranchs, TMAO is probably not significant in the osmoregulation of the marine teleosts. Since the marine prawns examined in this study are euryhaline at least during their early life their osmoregulatory mechanism is presumably different in some manner from that of the marine teleosts.

TMAO has been reported occurring in significant quantities in marine zooplankton[6] which often consists predominantly of small crustaceans, *i.e.*, copepods. Presumably the small crustaceans are capable of synthesizing the oxide from precursors though experimental evidence is still lacking, while in marine teleosts the origin of TMAO is essentially dietary. There appears to be sufficient indication for considering as an hypothesis that TMAO plays a more distinct rôle in the physiology of the prawns than in marine teleosts.

The significance of the difference in the TMAO content of the marine and backwater prawns respectively from the technological aspects is that the trimethylamine test for spoilage could be applied in the case of the former but it is of little value in the latter. The TMAO content of the backwater prawns, even when the salinity has reached the level of sea-water, is far less than that of the marine prawns (Table I). Further observations on the variation in the TMAO content of the backwater prawns in relation to the salinity changes are being continued.

The authors wish to express their thanks to Sri. M. Krishna Menon of the Central Marine Fisheries Research Station for the identification of the prawns used in these studies, and for helpful discussion.

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RELEASE OF 5-HYDROXYTRYPTAMINE ON STIMULATION OF NERVES TO FROG'S STOMACH

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The distribution of 5-hydroxytryptamine in the alimentary tract of dog has been described by Feldberg and Toh (1953). They found that the mucosa of the pyloric antrum and of the duodenum were regions of high concentration. Toh (1954) found that dog's stomach, perfused *via* the cœliac artery, releases 5-hydroxytryptamine spontaneously into the perfusate coming from the portal vein. 5-Hydroxytryptamine increases the motility of the intestines (Erspamer, 1952; Collier, 1957). It might be an intestinal hormone active in the digestive tract.

In the present research an attempt has been made to study the release of 5-hydroxytryptamine from frog's stomach on nervous and electrical stimulation.

Experimental

These experiments were performed on the stomach of the frog *Rana tigrina*. The stomach was dissected out with all its nerves attached to the lesser curvature. It was then split by cutting along the greater curvature. It was washed for half an hour in the same saline used for assaying 5-hydroxytryptamine on rat's uterus (de Jalon, Bayo and de Jalon, 1945). Frog's stomach muscle responds normally in such hypertonic saline (Singh, 1939); the rat's uterus saline is not in any way detrimental to the amphibian tissue. This was essential, otherwise it was not possible to assay the liberated 5-hydroxytryptamine.

After the preliminary wash the stomach was laid in a tilted petri dish containing 12 c.c. of rat's uterus saline for half an hour to study the spontaneous release of 5-hydroxytryptamine. Thereafter the nerves were stimulated for half an hour with induction shocks to study the release of 5-hydroxytryptamine by nervous stimulation. Lastly, immediately after nervous stimulation, it was stimulated directly with induction shocks, 16 times per minute in a small muscle chamber containing 12 c.c. of rat's uterus saline.

The various solutions were assayed for 5-hydroxytryptamine on the isolated atropinised oestrus uterus of the rat. A few similar experiments were also performed on dog's stomach.

Results

Most of the 5-hydroxytryptamine was found to come from the gastric mucosa; very little came from the muscle. This is in agreement with the finding that in the dog's duodenum, most of the 5-hydroxytryptamine is in the mucosa (Feldberg and Toh, 1953). In the dog the intestinal mucosa contains over 80 per cent. of the total body 5-hydroxytryptamine (Erspamer, 1954).

Table I

5-Hydroxytryptamine released from frog's stomach as a result of nervous and electrical stimulation

Number of experiment	5-Hydroxytryptamine in nanograms released from 1 g. of tissue in ½ hour		
	Resting	Nervous stimulation	Electrical stimulation
1	0	30	100
2	0	11	14
3	0	27	114
4	17	25	39
5	14	18	36
6	30	35	50
7	30	44	50
8	21	30	45
9	41	32	45
10	0	20	28
11	0	15	53
12	10	22	45
13	0	6	19
14	19	30	112
15	23	30	121
16	26	99	114
17	19	25	117

Out of 17 experiments on frogs there was spontaneous liberation of 5-hydroxytryptamine in 11 (Table I). Immediately after dissection, there may be a heavy liberation of 5-hydroxytryptamine, but the release ceases or the rate of release declines to a constant value in 15–30 minutes. Spontaneous liberation also takes place from isolated pieces of the mucosa of dog's

stomach from the pyloric region. In 3 such experiments, the release was 22, 19, 20 ng./g. of tissue per hour. This is in agreement with findings of Toh (1954) that dog's stomach releases 5-hydroxytryptamine spontaneously into the portal vein.

In frog's stomach, nervous stimulation released 5-hydroxytryptamine in all the 17 experiments, so also direct electrical stimulation. In dog's stomach, though there was spontaneous liberation, stimulation of the vagus or sympathetic, or direct electrical stimulation, did not show any significant effect. These results on dog's stomach are in agreement with those of Lee (1960) who found that the release of 5-hydroxytryptamine from the colon of guinea-pig and rabbit was not under the control of extrinsic nerves.

DISCUSSION

The presence of " entraminergic nerves in molluscs has been discussed (Florey and Florey, 1954). They found that injection of 1 μg. into a crab's claw causes muscular spasm. 5-Hydroxytryptamine might be a mediator substance in molluscs (Welsh, 1954, 1955). It might be an inhibitory mediator in *Mytilus edulis*; this muscle is extremely sensitive to 5-hydroxytryptamine but not to catechol amines (Twarog, 1954). The present experiments show that there are " tryptaminergic " fibres to the frog's stomach. The control experiments on dog's stomach show that these are neither cholinergic nor adrenergic. This is the first definite proof that nervous stimulation might release 5-hydroxytryptamine.

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TWO NEW SPECIES OF CHALCIDOID PARASITES FROM INDIA

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Received June 9, 1960

While surveying the citrus leaf miner infested orchards for parasitic and predatory insects, two species of parasites were collected. One of them, belonging to the genus *Cirrospilus* Westwood, was also reared in the laboratory on the citrus leaf miner *Phyllocnistis citrella* St. as an external parasite; the host for the other species, which belongs to the genus *Ceracterocerus* Mercet, is not known yet. The following is the description of the two new species.

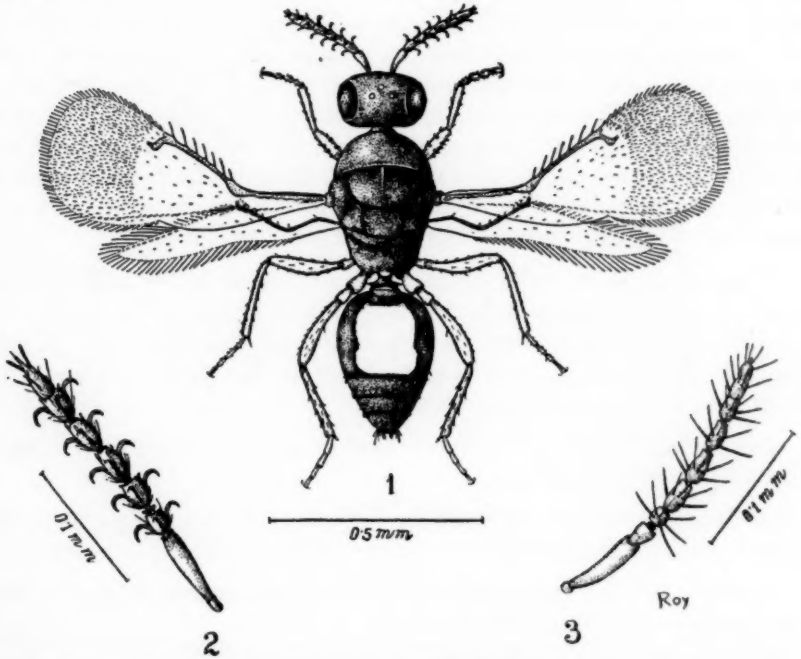

Fig. 1. 1. *Cirrospilus phyllocnistoides*, new species, female. 2. *C. phyllocnistoides*, new species, antenna, female. 3. *C. phyllocnistoides*, new species, antenna male.

Family: EULOPHIDÆ

Subfamily: *ELACHERTINÆ*

Tribe: ELACHERTINI

***Cirrospilus phyllocnistoides*, New Species**

Length.—1·15 mm.

Colour.—Brown to dark. Antenna pale yellow. Abdomen light brown with a clear hyaline patch occupying most of the tergal area of the first, second and third segments. Wings hyaline. Legs pale yellow to hyaline.

Head almost as wide as thorax. Vertex smooth shining except for a few very short bristles. Eyes moderately hairy. Ocelli arranged in a broad triangle, interocellar space slightly longer than the ocellocular spaces, the lateral ocelli one and a half times their own diameter away from the eye margin. Antennæ inserted in the middle of the face just above an imaginary line drawn joining the lower orbital borders. Antenna 9-segmented consisting of a scape, a pedicel, a ring joint, a 3-jointed funicle and a 3-jointed club. Scape long, more than twice as long as the pedicel; pedicel short, narrower and shorter than the first joint of funicle; ring joint very small, distinguishable only under high magnification in a well cleared and mounted specimen; first funicular joint shorter than the second; the second and the third almost equal, the club distinctly 3-jointed; the terminal segment ending in a short spine. The funicular and the club joints armed with four strong curved spines, perhaps very characteristic of the genus.

Thorax dark brown. Pro-thorax normal; scutum with a median longitudinal furrow, scutellum with two longitudinal furrows. Parapsidal furrows delicate. Wings hyaline; marginal vein slightly longer than the submarginal. Post-marginal vein absent. Stigmal vein about one-fourth of the marginal. Marginal fringe normal.

Legs.—All legs pale to hyaline. Hind tibia one and one quarter times as long as hind femur.

Abdomen.—Petiolate, very striking because of the hyaline patch on the tergum. Slightly shorter than the thorax. Ovipositor not exserted.

Male.—0·98 mm. long. Almost identical with female except for the strong straight bristles on the antennæ.

Described from a holotype female and an allotype male mounted on slide. A number of paratypes on slides (National Pusa Collection).

Host.—*Phyllocnistis citrella* Stainton.

Locality.—Delhi.

Collection.—B. R. Subba Rao, July 1954.

This species, though described under *Cirrospilus* Westwood does not agree in every respect with the genotype description. A new genus cannot be erected for this species on the not very significant generic characters. Hence I tentatively put the species under *Cirrospilus* Westwood.

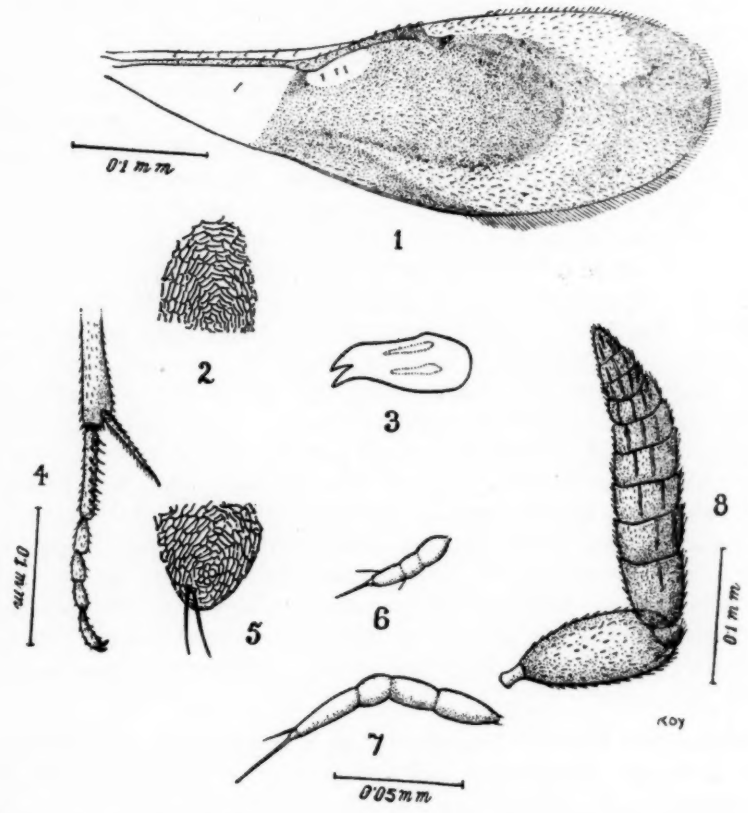

FIG. 2. 1. Fore wing of *Cerapterocerus antennatus*, new species, female. 2. Scutum sculpturing. 3. Mandible, female. 4. Middle tibia and spur, female. 5. Scutellum sculpturing. 6. Labial palpus, female. 7. Maxillary palpus, female. 8. Antenna, female.

Family: ENCYRTIDÆ

Cerapterocerus antennatus, New Species

General colour.—Honey-yellow to brown, abdomen being a shade darker than the rest of the body. Length 1·6 mm.

Female.—Head viewed from side longer than broad. Vertex very much narrowed, ocelli in an equilateral triangle, the posterior ocelli about their own diameter apart from the eye margins and about the same distance from the occipital margin. Eyes very large, orbits slightly divergent anteriorly. Antennal sockets large, their base tangent to the basal ocular line and originate close to the mouth border above the clypeus. Scrobes narrow and deep. Cheeks very short.

Scape large, leaf-like, light brown, almost equivalent to the combined length of the short pedicel and the first three funicular segments; in the funicle, the first and second are longer than broad; 3rd and 4th almost equal, 5th shorter than 4th, but longer than 6th but joints 3, 4, 5 and 6 are broader than long; the first joint of funicle is longer than the second. Club 3 segmented, rather indistinguishably separated from funicle. The antenna as a whole is characterised by its leaf-like structure. The number of sensoria and their situation are shown in Fig. 2, 8. Mandible distinctly bidentate. Maxillary palpus 4-jointed, the last joint ending in a short spine-like structure. Labial palpus 3-jointed. Prothorax relatively large. Mesonotum entire, scutum very much reticulated, recognisable only under high magnification; the pattern of reticulation is that of whorles of finger-prints; scutellum also finely reticulated but the pattern entirely different from that of scutum, the posterior margin armed with two long stiff bristles. Forewings dusky, with some hyaline patches. Marginal cilia short. Marginal vein is longer than stigmal, nearly two times that of stigmal. Post-marginal vein absent. Mesonotum narrow. Propodæum smooth and shining. Spiracular sulci round. Middle tibial spur almost as long as the first tarsal joint. Abdomen triangular, slightly shorter than the combined length of the head and thorax. Paratergites long and clearly demarcated; ovipositor very slightly exserted.

Male.—Not known. Described from a single specimen mounted on slide. Holotype deposited in the National Pusa Collection, I.A.R.I., New Delhi.

Collected by M. Ramachandra Rao, Delhi, February 1959.

Host.—Not known.

Man Mohan and Alam (1960) have presumably recorded a species of *Cerapterocerus* (spelt as *Ceraptocerus*). As there is only a summary of the description the present species could not be compared with *C. virens*. However, *in C. virens* they have described a short post-marginal vein which is absent in *C. antennatus* and in *C. virens* the hind femur is blackish brown, whereas in *C. antennatus* the hind femur is lemon-yellow.

Reference

Man Mohan and Alam, S. M. .. "New Coccid inhabiting Encyrtid parasites recorded from Aligarh," *Proc. 47th Ind. Sci. Cong.*, 1960, Part III, p. 457.

THE MALE AND FEMALE GAMETOPHYTES OF *PASSIFLORA FOETIDA* LINN.

By M. D. Padhye and B. G. Deshpande*

(*St. Francis de Sales' College, Nagpur*)

Received August 29, 1960

(Communicated by Dr. T. S. Mahabale, F.A.SC.)

Introduction

The Passifloraceæ—a small family of herbs and shrubs—is included in the Parietales along with thirty other families of the Polypetalous angiosperms (Lawrence, 1951). It consists of twelve genera and about five hundred species (Rendle, 1952) distributed over the warmer parts of the world but is chiefly confined to Tropical America. In India it is represented by three genera and eleven species (Hooker, 1885). A study of the previous literature reveals that some species of the genus *Passiflora* have not been studied embryologically. Recently Raju (1956) has worked out the embryology of *Passiflora calcarata*. But the other Indian species are still unworked. It was, therefore, decided to study the life-history of *Passiflora fœtida* Linn., an annual herb found growing on hedges in many parts of India. The previous literature on the embryology of the family is reviewed by Schnarf (1931) and recently by Raju (1956) and therefore it is not summarised here.

Material and Methods

The material was collected locally and fixed in formalin-acetic alcohol and Navaschin's fluid. It was washed directly in 70% alcohol. Dehydration and embedding was done according to the customary methods. Sections were cut varying from 8–12 μ thick. Heidenhain's hæmatoxylin was used as stain. The slides were destained in a saturated solution of picric acid. The external characters of the pollen grains were studied after mounting them in methyl-green glycerine jelly.

Microsporangium and Microsporogenesis

The male archesporium is a plate of hypodermal cells. It comprises two or three cells in a transverse section and extends over the entire length

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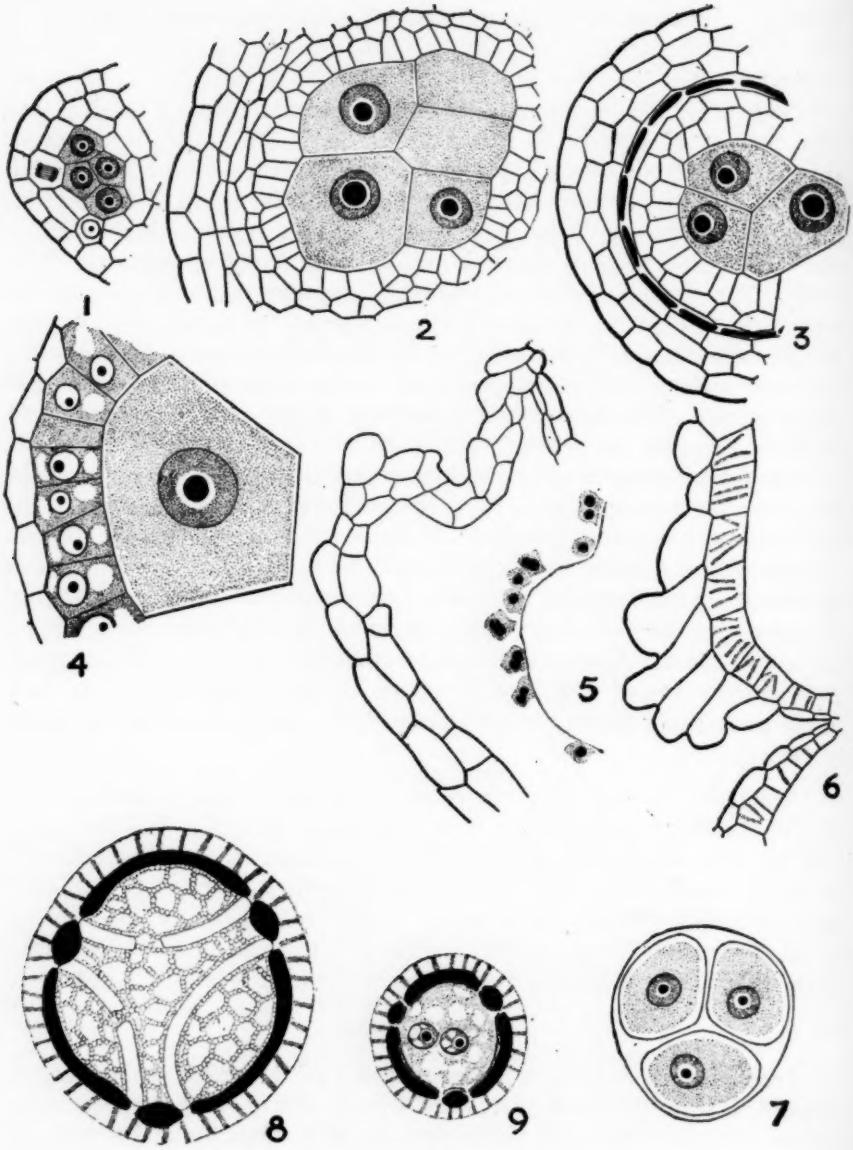
of the anther lobe. The archesporium cuts off a parietal layer (Fig. 1) which undergoes repeated divisions in the periclinal and anticlinal planes forming ultimately five layers of the parietal tissue excluding the epidermis (Figs. 2, 3). The outermost parietal layer occupying the hypodermal position functions as the fibrous endothecium (Fig. 6). The innermost layer develops into the tapetum, while the remaining three layers degenerate during development. The process of degeneration begins in the fourth layer from outside (Fig. 3). The cells of the tapetum become radially elongated and while doing so they become vacuolated also (Fig. 4). Their nucleus divides forming a binucleate tapetum of the glandular type. It is mostly uniseriate but at places it becomes biseriate at certain regions as in *Passiflora calcarata* (Raju, 1956). The method of degeneration of the tapetum is, however, somewhat unusual in this species. Generally in angiosperms the degeneration in tapetal cells affects the cytoplasm, cell-wall and the nucleus almost simultaneously but in *Passiflora fœtida* the degeneration occurs in stages. To begin with, the cytoplasm of the tapetal cells is gradually consumed and the radial walls begin to disappear. The nuclei, embedded in a little amount of cytoplasm, are thus set free in pairs which continue to remain in healthy condition at the periphery, for a comparatively long time unlike in other angiosperms. The cell-walls on the inner tangential side, however, do not disappear simultaneously with the radial walls but persist for sometime forming a sort of ephemeral septum between the pollen grains and the anther wall (Fig. 5). Ultimately, however, the nuclei along with the cytoplasm and septum degenerate and disappear. Such a degeneration of the tapetal cells in stages and the formation of an ephemeral septum out of the inner tangential walls of the tapetal cells form quite interesting features in the anther development of this species.

The pollen mother cells undergo an enormous increase in size becoming four or five times bigger than their original dimensions (Fig. 4). The nuclei also become very much enlarged. The cytoplasm increases in quantity. As usual it recedes from the cell-wall and becomes surrounded by the mucilaginous sheath. Finally each pollen mother cell undergoes two meiotic divisions forming four pollen grains. The arrangement of the young pollen grains is usually tetrahedral (Fig. 7) but sometimes it is isobilateral also.

Pollen Grains

Soon after its formation the tapering end of the pollen grain becomes rounded off when the microspores are still enclosed within the common mucilaginous sheath. The young microspore is rich in cytoplasm and is enclosed by the intine and exine. The former is a delicate membrane. The latter is



FIGS. 1-9

divided into sexine and nexine (Figs. 8, 9). The sexine is reticulate and is composed of closely set radially arranged bacula enclosing faintly staining polygonal areas. The bacula support a thin roof and in surface view have a circular appearance simulating a tubular structure. Unlike the sexine, the nexine is very much thickened into which the bacula of the sexine extend up to a certain distance. No rods or granules are present in the lumina of the reticulate sexine as in *Passiflora maculifolia* (Erdtman, 1952). The pollen grains are typically hexacolporate unlike those of *Passiflora maculifolia*, where they are 12-colporate (Erdtman, 1952). The six colpi are arranged in three groups of two each. The colpi are free from each other in the beginning (Fig. 8) but later on they have a tendency to fuse in pairs by their polar ends presenting a band-like or bangle-like appearance.

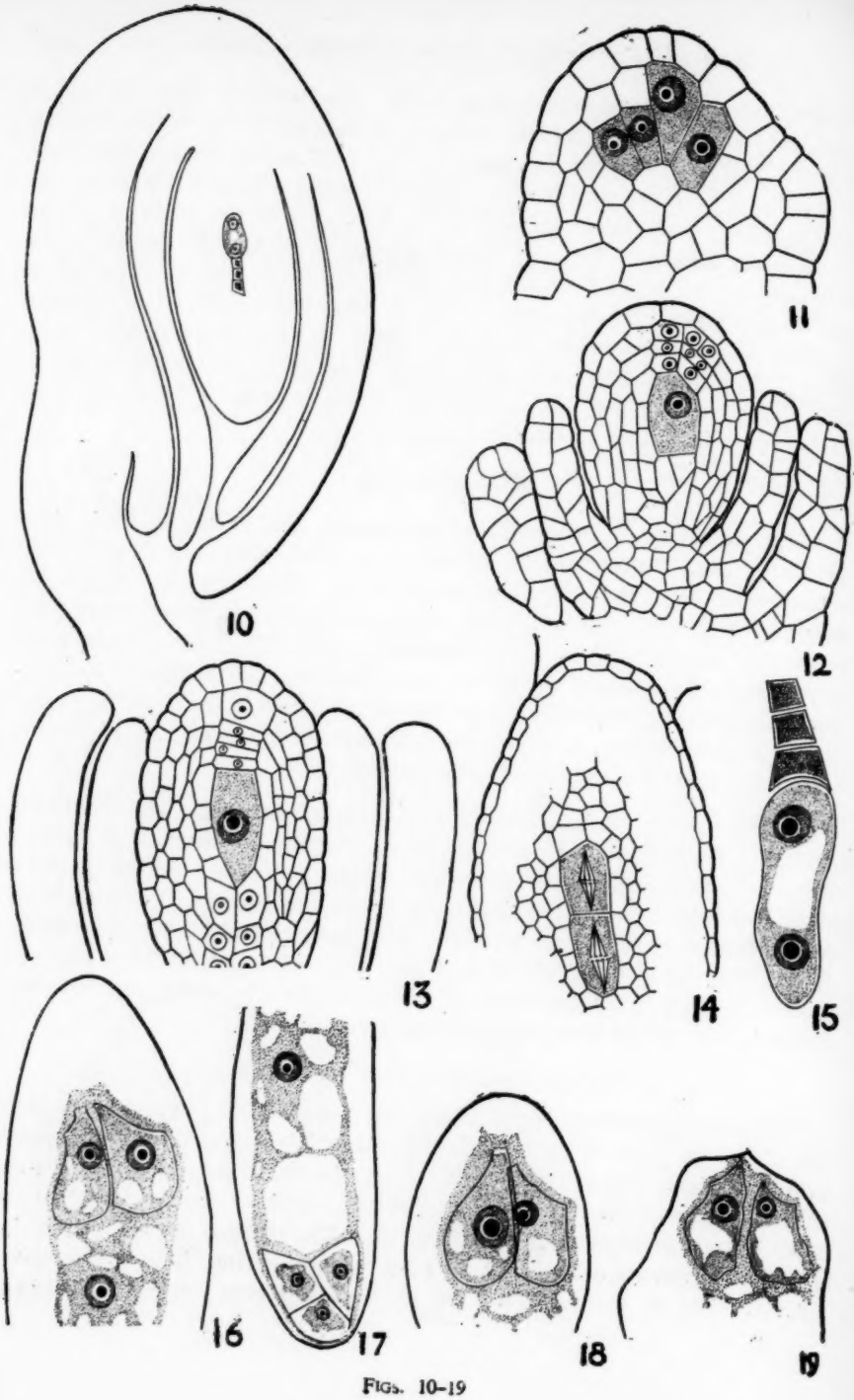
Megasporangium

The gynœcium is tricarpellary, syncarpous and unilocular with three placentas, each bearing several ovules. The ovule develops as a massive protuberance from the upper part of which the initials of the integuments originate (Fig. 11). The ovules are crassinucellate, bitegmic and anatropous as in other members of the family (Schnarf, 1931). These integuments close over the nucellus at about the tetrad stage (Fig. 10). At the time of fertilization both the integuments consist of four or five layers in the chalazal region. In the micropylar part, however, the inner integument consists of two layers while the outer one consists of three layers.

The formation of the micropyle deserves some mention. The endostome as usual is formed by the inner integument. But the organization of the exostome is somewhat different. The inner integument on the side of the funiculus protrudes beyond the outer integument and, therefore, on this side as seen in section, the exostome is formed by the inner and the outer integuments (Fig. 10), unlike in other members of the Parietales in which both the integuments take part in the formation of the micropyle. No epidermal caps are formed as in *Bergia ammanioides* (Kajale, 1940).

Megasporogenesis and Embryo-Sac

The female archesporium consists of two to four hypodermal cells (Fig. 11). Only one of them develops further while the remaining cells merge into the nucellar tissue. The functioning archesporial cell cuts off a parietal cell and becomes the megaspore mother cell which at times is mounted on a double row of supporting cells (Fig. 13). The primary parietal cell in the beginning divides mostly in the periclinal plane forming about five to seven parietal cells in a row (Fig. 13) at the megaspore mother cell stage.



FIGS. 10–19

Sometimes, however, it first divides anticlinally and then there are two rows of parietal cells over the megaspore mother cell (Fig. 12). These cells undergo further divisions as a result of which the tetrad and later the embryo-sac becomes deep-seated. Fifteen to twenty parietal layers are formed at the time when the embryo-sac reaches maturity. The megaspore mother cell divides to form two dyad cells which are either equal in size or the upper one may be slightly smaller. The second division forms a quartet of megaspores (Fig. 14). The upper three megaspores degenerate early but sometimes their remains persist up to the 2-nucleate stage of the embryo-sac (Fig. 15). The chalazal megaspore develops into the embryo-sac of the Polygonum type (Maheshwari, 1950). The synergids are pear-shaped and hooked. They have a basal vacuole with a nucleus above it (Figs. 16, 18, 19). The egg is flask-shaped. The vacuole may or may not be present in it in the micropylar part. Sometimes a few vacuoles may be seen below the nucleus also (Fig. 18). The nucleus of the egg is slightly bigger than that of the synergid (Fig. 18).

The antipodals are represented by three cells which occupy the chalazal end of the embryo sac (Fig. 17). They persist for a short time. There are two polars and their size is bigger than the size of the nuclei in the antipodals (Figs. 16, 17).

Summary

The paper deals with the male and female gametophytes of *Passiflora fœtida* Linn.

The male archesporium is multicellular and cuts off a parietal layer. The anther wall consists of the epidermis, fibrous endothecium, early degenerating three middle layers and the secretory tapetal layer of binucleate cells. The pollen grains are hexacolporate. The colpi are present in three groups of two each. They fuse in pairs at the poles describing a belt or bangle. The exine consists of reticulate sexine and thickened nexine. The pollen grains are shed at the two-celled stage.

The ovary is tricarpellary, syncarpous and unilocular. The ovules which are borne on the parietal placentas are bitegmic, crassinucellate and anatropous.

The female archesporium consists of three to five hypodermal cells. The parietal cell is cut off. The embryo-sac development conforms to the Polygonum type. The egg is flask-shaped with a nucleus at the base and a vacuole above it. The synergids are pear-shaped and hooked. The vacuole is at the base. The antipodals are three small ephemeral cells.

ACKNOWLEDGEMENTS

Our grateful thanks are due to Dr. L. B. Kajale, Principal, Government Science College, Nagpur, for helpful guidance and criticism. Thanks are also due to Prof. T. S. Mahabale for his kind interest in our work. The senior author is grateful to Father M. M. Balaguer, S.J., Principal, S.F.S. College, Nagpur, for facilities and encouragement.

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EXPLANATION OF FIGURES

FIGS. 1–9. *Passiflora fætida* Linn. Figs. 1–3. T.S. of anther lobes showing stages in the development of anther wall; note the degeneration starting in the fourth layer shown in Fig. 3. Fig. 4. Microscope mother cell with tapetal cells. Fig. 5. A part of the anther lobe in T.S. showing degeneration of the tapetal cells. For details see text. Fig. 6. T.S. of anther wall showing fibrous endothecium. Note the radially enlonged epidermal cells on one side near the line of dehiscence. Fig. 7. Tetrahedral arrangement of the young pollen grains. Fig. 8. The surface and optical view of the mature pollen grain. Note the reticulations, colpi and two layers of exine. Nexine is shown in black. Fig. 9. Two-celled pollen grain [(Figs. 6, ×250) (Figs. 1–3, 5, 7 and 9, ×500 Figs. 4 and 8, × 900).]

FIGS. 10–19. *Passiflora fætida* Linn. Fig. 10. L.S. ovule showing organisation of the micropyle. Fig. 11. L.S. apex of young ovule showing two archesporial and two megaspore mother cells. Figs. 12–13. L.S. apex of young ovule showing megaspore mother cells and the formation of the parietal tissue. Note the double row of supporting cells below the megaspore in Fig. 13. Fig. 14. L.S. apex of nucellus showing meiosis II in dyad cells. Fig. 15. 2-nucleate embryo-sac with three degenerating megaspores. Fig. 16. Micropylar part of the embryo-sac showing two synergids and the micropylar polar nucleus. Fig. 17. Lower part of the embryo sac showing three antipodals and the lower polar nucleus. Fig. 18. Micropylar part of the embryo-sac showing egg and synergid. Fig. 19. Same as above showing two synergids [(Fig. 10, ×250); (Figs. 12–14, ×500); (Figs. 11, 15–19, ×900).]

THE EMBRYOLOGY OF *AVICENNIA OFFICINALIS*

I. Floral Morphology and Gametophytes

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INTRODUCTION

A CRITICAL appraisal of the available embryological literature on the Verbenaceæ indicates that our knowledge is fragmentary even in regard to those of the taxa that have been investigated. Furthermore, controversial opinions have been expressed in regard to the systematic position of *Avicennia*. Although a detailed discussion of this topic will be taken up in the final paper of this series, it may be briefly mentioned here that a school of taxonomists have ventured to create an independent family, the Avicenniaceæ, to accommodate this genus. This position has been implemented solely on the basis of exomorphic characters. Therefore it is desirable to collect and collate arguments either in refutation or in support of the existing differences of opinion in regard to the taxonomic status and relationships of *Avicennia*.

The earliest published account on the embryology of the verbenaceous taxa dates back to the year 1858 when Hofmeister recorded his observations on *Verbena officinalis*. This was soon followed by the publications of Warming (1873) and Jonsson (1879-80) on *Verbena* and Treub (1883) and Karsten (1891) on *Avicennia officinalis*. Observations on the development of the embryo and endosperm (in a rather casual manner) in *Tectona grandis* were published by Koorders in 1896. The next contribution appeared twenty-five years later when Kanda (1920) reported his observations on species of *Verbena*. This paper, by virtue of its unusual contents, created a mild controversy in which Dahlgren (1923) and Schnarf (1925) also expressed their opinions. A summary of embryological literature on Verbenaceæ has been published by Schnarf (1931).

Dissertations of Schwencke (1931) and Patermann (1935) contain more detailed studies on a number of verbenaceous taxa, the results of which are important additions by way of factual matter. Junell's (1934) work is also broad-based, incorporating a number of genera and species, but the information gathered is rather fragmentary.

Other contributions are by Misra (1939) notably on *Clerodendron phlomidis*, Tatachar (1940) on *Lantana indica* and *Stachytarpheta indica*, Pal (1951) on *Lippia nodiflora*, *Tectona grandis* and *Vitex negundo* and Maheshwari (1954) on *Lippia nodiflora*. Recently Padmanabhan (1959) has shown that Tatachar's report of the development of antipodal haustorium in *Lantana* is misleading. Treub's (1883) contribution stands as the earliest observation on the female gametophyte of *Avicennia*. He demonstrated the possible existence of a bisporic type of development in this genus. Karsten (1891) also regarded that the development of the female gametophyte followed the Allium type. Junell (1934) confirmed the existence of a bisporic development of the female gametophyte in *Avicennia*. The rather ambiguous statement of Treub on the bisporic development of the embryo-sac, and the inclusion of the female gametophyte of *Avicennia officinalis* under the bisporic type (Maheshwari, 1955) prompted the present investigation.

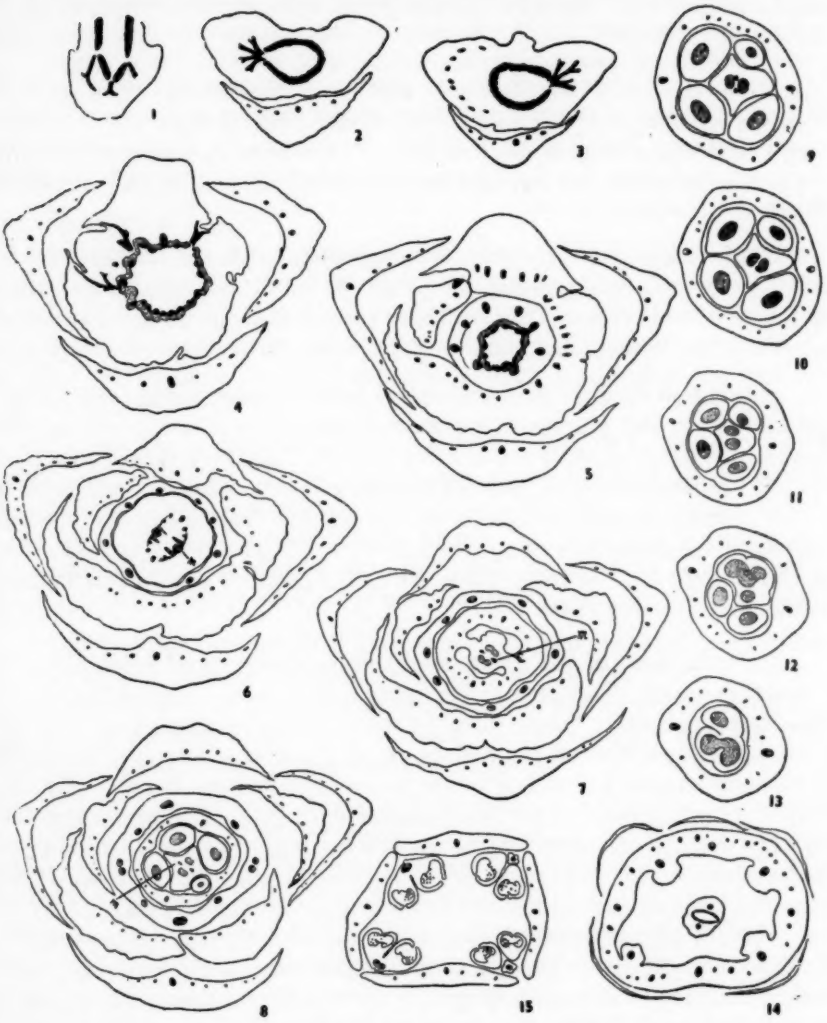
OBSERVATIONS

Floral vasculature.—The first primordium to arise on the floral apex following those of the bracteoles is that of the calyx. Next, the stamen primordia are initiated. The petal primordium becomes initiated almost simultaneously at the base of the staminal primordia. The initiation of the gynœcium takes place last of all. Toral growth predominates in the primordium of the gynœcium, as a result of which, an urceolate depression is soon organised. The rim of the cup being highly meristematic begins to grow rapidly whereby the style and stigma become differentiated. Simultaneously with these changes, the apex of the floral axis grows into the placenta. The terminal portion of the placental column gives rise to four ovule primordia. The ovules grow down and become pendant. The order of development of the floral whorls is as follows: calyx, stamens, corolla, gynœcium.

The vascular supply to the bract is given out by the stele of the peduncle. Each bract is vascularised by three strands, one median and two laterals. These are related to three independent gaps (Fig. 1). The two bracteoles, which are somewhat opposite to one another, arise at slightly successive levels on the floral axis (Figs. 2, 3). Each bracteole is supplied with three strands, all arising from a single gap (Figs. 2, 3). Above the level of the bracteoles the vascular strands form a complete ring as seen in a cross-section.

The sepals appear at slightly different levels (Figs. 4–6). Each sepal is vascularised by one median bundle and two laterals. The lateral strands of adjacent sepals arise from commissural bundles (Fig. 4).

Above the level of the sepaline vasculature the axial cylinder gives off strands to the petals and stamens (Fig. 5). The petals and stamens each



FIGS. 1–15. Cross-sections of the flower at successive higher levels showing vascular patterns. 1–3, ×20; 4–8, ×10; 9–13, ×6; 14–15, ×5.

receive a single strand. Because the stamens are adnate to the corolla tube, the four bundles supplying them lie in a ring alternating with the four petal bundles (Fig. 6). Higher up, the staminal bundles depart from the ring and enter the filaments, ultimately ending in the connective (Fig. 15). The median

bundle of each petal branches repeatedly during its upward course (Figs. 7, 8, 14, 15).

Immediately after supplying the petals and stamens the vascular tissue of the axis forms a closed ring. Each carpel receives a prominent midrib bundle (primary dorsal) (Figs. 6–8, D). Several smaller bundles also supply the ovary wall (Fig. 7). The primary dorsal bundles run through the whole length of the style (Fig. 14).

Besides supplying vasculature to the gynœcial wall, the axial system vascularises the placental column also (Figs. 6, 7). Thus, two bundles occur in the structure (Figs. 6, 7 m.). These strands correspond to the ventral bundles even though their real nature is difficult to interpret.

In addition to these primary ventral bundles which exhibit fully differentiated xylem and phloem, the placental column receives four procambial strands, which arise in opposite pairs (Fig. 6). Each of these procambial strands supplies one ovule, whereas the two primary bundles—corresponding to the primary ventral strands—mentioned above fade out in the terminal regions of the placental column (Figs. 7–11). The procambial strands at the place of origin are relatively thinner but during their upward course become considerably thicker (Figs. 6–11).

The behaviour of the procambial strands during their upward course is rather peculiar. Soon after their origin the strands fuse in pairs (Figs. 7, 8). The fusion always takes place between strands belonging to opposite pairs. A cross-section of the column cut at the level of fusion exhibits two procambial strands situated opposite the primary ventral bundles (Fig. 8). Higher up in the column the fused strands undergo a twisting through 180 degrees—much in the same way as the phloem strands of some seedlings at the transition region—and come to lie very closely along the outer side of the respective primary ventral bundles (Figs. 9–11). Ultimately, they appear to fuse with the primary ventrals and a cross-section through the extremity of the column shows two prominent procambial strands situated in the plane of the primary ventral bundles (Fig. 11). Even before reaching the very apex of the placental column, the primary ventrals fade out (Figs. 10, 11). Occasionally one or two tracheary elements belonging to them could be identified in cross-sections of the top of the column. The fused procambial strands bifurcate in a plane at right angles to the primary dorsals and supply the ovules (Figs. 12, 13). Thus, even though the primary ventrals do not directly supply the ovules, there seems to be a certain amount of fusion of these strands with the procambial strands.

Microsporogenesis and male gametophyte.—Four groups of hypodermal archesporial cells are seen in a cross section of a four-lobed anther primordium. Each group consists of a plate of cells as seen in transections. In longisections the archesporium appears as a row made up of several cells (Fig. 16). The archesporial cells cut off the parietal layer, which, in turn, divides in the periclinal plane so as to form three to five wall layers; the outermost of these later forms the endothecium and the innermost the tapetum (Fig. 17).

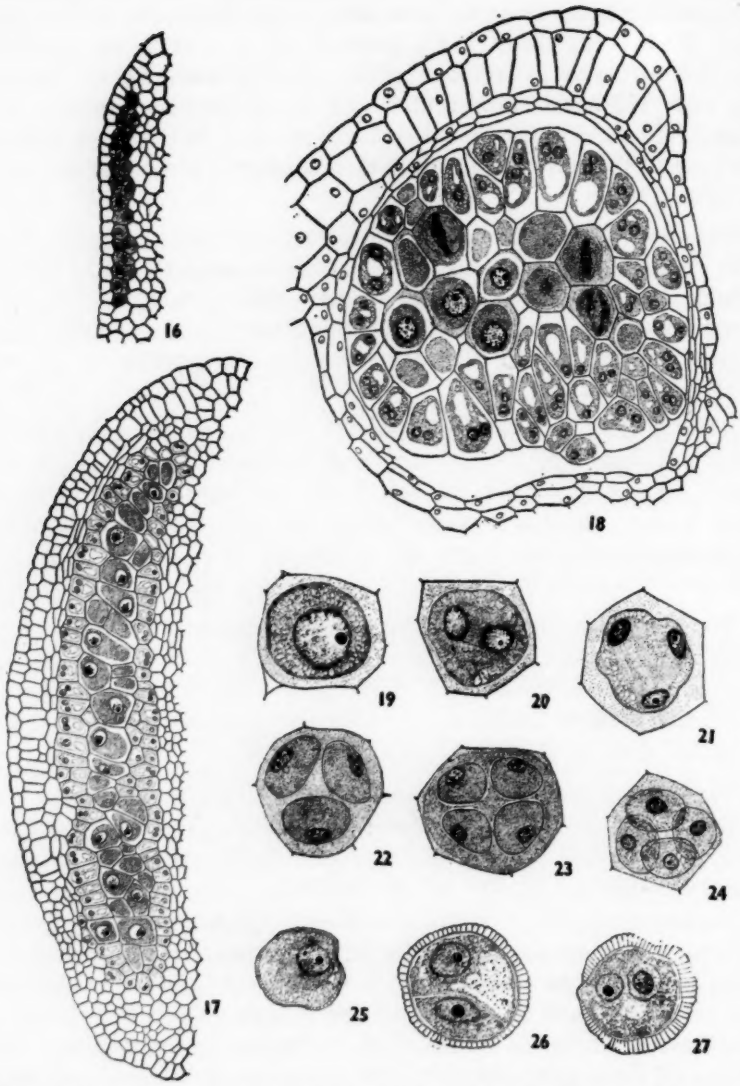
The cells of the endothecial layer elongate slightly in radial direction before developing the characteristic fibrous thickenings (Fig. 18). By the time of the first meiotic division in the pollen mother cells, all the tapetal cells become binucleate (Fig. 18). The tapetum functions in a secretory manner; the inner wall of the cells exhibits minute granular markings at the time of microspore formation.

After the first meiotic division no cell plate is formed in the microspore mother cell (Figs. 19, 20) and the daughter nuclei begin to divide almost immediately. At the end of meiosis II the resulting four microspore nuclei appear to be disposed in a tetrahedral manner (Fig. 21) although tetragonal and decussate arrangements are also frequently encountered (Figs. 22–24). Quadripartition of the mother cell is brought about by the furrowing method.

The microspore nucleus (Fig. 25) moves to one end of the cell and divides to give rise to a lenticular generative cell towards the wall (Fig. 26). Later the generative cell loses contact with the spore wall and comes to lie without any definite position within the cytoplasm of the vegetative cell (Fig. 27). The pollen grain is shed at the two-celled stage.

Megasporogenesis and female gametophyte.—According to taxonomic descriptions, the ovary is imperfectly four-celled. The ovules are orthotropous and unitegmic. The central column is four-winged (Fig. 9). The ovules are suspended in between the wings.

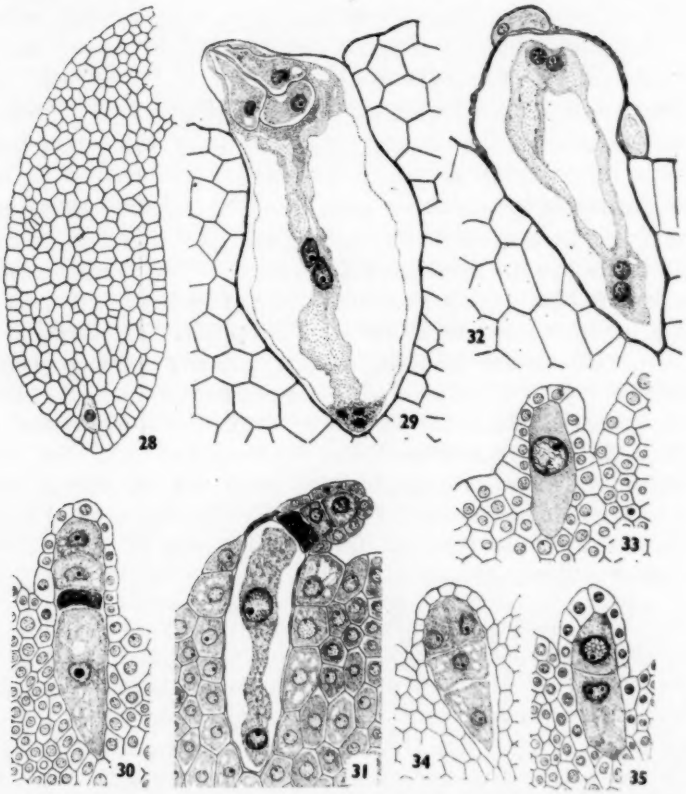
The structure of the ovule in *Avicennia officinalis* appears to deviate from the norm characteristic of the family Verbenaceæ. In an ovule primordium in which the archesporium is already differentiated, neither integuments nor the limits of the nucellus can be recognised (Fig. 28). Even during later stages, the structures are not clearly definable. From the time of differentiation of the megaspores, however, the tissue of the ovule surrounding the linear tetrad becomes somewhat raised. At this stage the free part of the tetrad is enveloped by a single layer of epidermis (Fig. 30) which should be interpreted as nucellar. The raised tissue perhaps should represent the arrested integument. With maturation of the female gametophyte the



FIGS. 16–27. Fig. 16. Longisection of young anther showing archesporium, ×334. Fig. 17. Longisection of anther at spore mother cell stage, ×214. Fig. 18. Transaction of an anther locule at the time of onset of meiosis in the spore mother cells, ×234. Figs. 19–27. Stages in cytokinesis in pollen mother cells and formation of pollen grains, ×1,200.

nucellar epidermis becomes disorganised (Fig. 32) and the micropylar end carrying the egg-apparatus lies exposed within the cavity of the ovary (Fig. 29).

The archesporial cell functions directly as the megaspore mother cell. The latter elongates considerably before embarking on the first meiotic division (Fig. 33). This division is followed by the formation of a wall between the nuclei. The two dyad cells (Fig. 35), thus formed, divide again to give rise to a linear tetrad of megaspores (Fig. 30). Occasionally T-shaped tetrads are also formed (Fig. 34). The chalazal megaspore continues further development (Fig. 31).



FIGS. 28–35. Fig. 28. Long section through nucellus with archesporial cell. Fig. 29. Mature embryo-sac. Fig. 30. Megaspore tetrad. Fig. 31. Two-nucleate embryo-sac with persisting megaspores. Fig. 32. Four-nucleate embryo-sac. Fig. 33. Megaspore mother cell. Fig. 34. T-shaped tetrad of megaspores. Fig. 35. Dyad stage. Figs. 28–35, ×500.

The third megaspore from the free end is smallest in size and disintegrates early (Figs. 31, 36). The two micropylar megaspores germinate to varying stages and persist for a long time. But they are pushed apart during the later stages when the embryo-sac grows out of the ovule and protrudes into the locule.

The nucleus of the functional megaspore undergoes three successive divisions to give rise to eight nuclei of the mature embryo-sac. At the four-nucleate stage, the embryo-sac protrudes beyond the ovule and gains direct access to the locule (Fig. 32). The micropylar quartet of nuclei becomes organised into a pair of synergids, an egg cell and a polar nucleus. Of the four nuclei at the chalazal end three undergo precocious disintegration whereas the fourth migrates towards the opposite pole and lies in contact with its homologue. Thus, the embryo-sac at the time of fertilization (Fig. 29) possesses only the egg apparatus and the juxtaposed polar nuclei.

DISCUSSION

Floral morphology and floral anatomy.—The sequence of development of floral appendages in *Avicennia* follows the same order as in the other members of the family investigated so far (Pal, 1951; Maheshwari, 1954). In these species, the sepaline primordia differentiate first. Next, the stamen primordia originate followed by those of the petals. The carpel primordia appear last. The stamens and petals originate from a common primordium. Kanda (1920) reported that in *Verbena* the stamens are the first to appear on the floral axis. But, this seems to be doubtful as pointed out by Pal (1951). In *Tectona grandis* (Pal, 1951) the central axis elongates after the initiation of the carpel primorida and gives rise to lateral placentas bearing four pendulous ovules. In *Avicennia* also, the central axis itself elongates as the placental column from the free end of which ovules are attached in a suspended manner.

Floral vasculature.—Apart from the publications of Junell (1934), Saunders (1939) and Rao (1952) the floral anatomy of the Verbenaceæ is unknown. Junell (1934) worked on three species of *Avicennia—A. officinalis*, *A. marina* var. *alba* and *A. nitida*. He has dealt only with the morphology of the gynœcium whereas Rao (1952) gives a complete account including the behaviour and course of vasculature. A critical appraisal of Junell's illustrations indicates that the gynœcium morphology of all the three species remains more or less stereotyped. According to him, the ovary is four-chambered at the base due to the development of the false septum and at higher levels it becomes unilocular.

Rao (1952) investigated the vascular anatomy of the flower of *Avicennia officinalis*. Although his account is fairly complete and conclusive in regard to the calycine, corolline and androecial whorls, the treatment of the gynœcium is rather inadequate. According to Rao's observations, the vascular traces of the axis form a ring at the base of the gynœcium; the ring gives off two prominent bundles to the ovary wall. These are considered to be the dorsal bundles of the carpels; besides the dorsal bundles, a number of smaller bundles enter the ovary wall and branch profusely; the placentum receives four strands which remain procambial throughout; "of the four placental bundles, the two belonging to each carpel fuse early so that only two placental bundles are derived"; higher up, the two placental bundles fuse into a single trace which again breaks up into four ovular traces. The present investigation, however, has brought out the occurrence of two more bundles in the placental column. These bundles possess completely differentiated xylem and phloem. They originate from the vascular ring at the base of the gynœcium itself on opposite radii. Two new procambial strands arise from either side of these bundles. In the terminal regions of the placental column the median bundles fade out while the newly formed lateral strands continue beyond the level of the median bundle. The behaviour of the four persisting procambial strands is interesting. After emerging from the vascular ring, the strands fuse in pairs and come to lie opposite to each other in a line with the dorsal bundles. Higher up, the fused strands undergo a sort of torsion and come to lie on the outer side of the median bundles. Now, the strands are oriented in a plane at right angles to that of the dorsal bundles. The change in orientation of these bundles is evident in Rao's illustrations, even-though he mentions nothing about this phenomenon in the text. The two fused strands lie very close to each other but do not merge as shown in Rao's illustrations (Fig. 62, p. 310). Furthermore, a comparison of his Figs. 61 and 63 clearly shows that the placental procambial strands in Fig. 61 are aligned in the same plane as that of the two dorsal bundles whereas in Fig. 63 they are oriented at right angles to the dorsals. This change in orientation is brought about by the peculiar torsion of the strands. The significance of the two median bundles in the placental column, which, however, disappear at the subterminal levels without supplying th eovules is unknown. In *Tectona grandis*, Rao (1952) mentions the existence of such bundles in the placental column. He interprets that the presence of such bundles may indicate an ancestral tetracarpellary condition of the gynœcium.

Microsporogenesis and the male gametophyte.—Although detailed account of development is restricted to a handful of species in the large family Verbenaceæ, the structure of the anther and the development of the microgameto-

phyte appear to follow a fairly uniform plan. The simultaneous method of meiotic division in the microspore mother cells, the tricolpate nature of pollen grain and the two-celled shedding stage appear to be common features of all species studied until now. In *Verbena*, according to Kanda (1920) and Schwencke (1931), the spindles during second meiotic division align themselves in a cross-wise manner. Patermann (1935) records a parallel alignment of the spindles in all the representatives studied by him and a similar situation has been described by Maheshwari (1954) in *Lippia nodiflora*. In *Avicennia officinalis*, there does not appear to be any uniformity in regard to the alignment of the spindles; tetragonal, tetrahedral and decussate arrangements being most frequent often within the same anther locule. Casual observations suggest that the different types of configurations within the same locule are to some extent dependent upon the location of the developing pollen mother cells.

Avicennia officinalis falls in line with the other members of the family in respect to the behaviour of the tapetum. Patermann (1935) records the wandering of the tapetal cells to varying distances within the locule, however, not constituting a periplasmodium. In all other members of the family the tapetum is of the secretory or glandular type and the cells become binucleate or tetranucleate. *Avicennia officinalis*, as also in *Canadea aubletia*, *Lantana triflora*, *Stachytarpheta cayennensis*, *Priva lappulacea*, *Duranta plumeri*, two species of *Clerodendron* (Patermann, 1935) and *Verbena angustifolia* (Kanda, 1920) exhibit the former condition, while *Verbena officinalis* (Schwencke, 1931), *Callicarpa pedunculata*, *Premna integrifolia* (Patermann, 1935) and *Lippia nodiflora* (Maheshwari, 1954) exhibit the latter condition.

In all members of the family, the shedding stage of the pollen grain takes place at the two-celled condition. As observed by Maheshwari (1954) Junell's report of a three-celled condition in *Stachytarpheta dichotoma* needs confirmation.

Megasporogenesis.—The members of the Verbenaceæ are characterised by unitegmic anatropous ovules. The configuration of the ovule of *Avicennia officinalis* has been stated to be orthotropous in many taxonomic descriptions (Moldenke, 1946). A study of the developmental stages appears to indicate that this condition is only apparent and similar studies in other members of the family confirms this. In all members of the Verbenaceæ, wherein studies have been carried out (*see* particularly Junell, 1934), the primordium of the ovule originates in an erect position, more or less at right angles to the placenta. Due to a pronounced unilateral growth, the ovule soon assumes an anatropous configuration. In other words, the ovule exhibits during its growth, a curvature of nearly 180 degrees. In *Avicennia officinalis* as also

in several other members of the family (Junell, 1934) the primordium of the ovule originates in the same way but the quantum of unilateral growth is not seen to be as extensive as in the other members. Thus, the ovule primordium undergoes a curvature of only about 90 degrees, so much so, the apex of the ovule lies somewhat adjacent to the base of the placental column. This situation certainly simulates an anatropous configuration, but in reality, this condition should be interpreted more as an arrested stage in the development of an anatropous ovule.

In all members of the Verbenaceæ, so far studied, the amount of nucellus is reduced and becomes absorbed at an early stage. Kanda (1920) reported the presence of a persisting nucellar cap in *Verbena*. This has been contradicted by Schwencke (1931). The nucellar epidermis is absorbed at an early stage in *Avicennia officinalis* also.

The hypodermal archesporium is single-celled in most of the Verbenaceous genera. Recently, two-celled archesporium has been found in *Lippia nodiflora* (Pal, 1951). The archesporial cell functions directly as the megaspore mother without cutting off a parietal cell. The nucellus is usually composed of one layer of cells. Occasionally in *Lippia nodiflora* (Maheshwari, 1954) one or two cells of the nucellar epidermis may divide periclinally. Such a feature, however, was not seen in *Avicennia officinalis.*

Even though linear tetrads of megaspores are commonly formed, T-shaped tetrads occur in the species of *Verbena* (Schwencke, 1931). In *Avicennia* linear tetrads represent the general condition and T-shaped ones the occasional condition.

Although, as a rule, the mature gametophyte is developed by the chalazal megaspore, the germination of more than one megaspore of a tetrad to varying successive stages is known in some members of the family. For example, Junell (1934) has figured two megaspores developing simultaneously in *Bouchea incrassata.* Persistent non-functional megaspores have also been recorded in *Petræa volubilis* (Junell, 1934) and *Citharæxylum ilicifolium* (Patermann, 1935). *Avicennia officinalis* resembles these examples.

Female gametophyte.—The embryo-sac development in the members of the Verbenaceæ so far investigated conforms to the monosporic eight-nucleate type. However, an Allium type of development was reported in *Avicennia officinalis* (Karsten, 1891) and *Avicennia marina* (Junell, 1934). Even though Treub (1883) described the development of the embryo-sac in *Avicennia officinalis,* his account seems to be involved in ambiguity. P. Maheshwari (1937) expressed doubt over the reports of Karsten and

Junell, mentioning that a reinvestigation was necessary to confirm the bisporic origin of the embryo-sac of *Avicennia*. In a recent review, of bisporic embryo-sacs of angiosperms, S. C. Maheshwari (1955) included *Avicennia officinalis* and *A. marina* in the list of plants in which bisporic origin of embryo-sac has been established beyond doubt, referring to the works of Karsten (1891) and Junell (1934).

It may be observed that, Junell (1934) failed to make detailed observations on the embryo sac development in *Avicennia marina* and his conclusions are not supported by clear illustrations. He mentions that since he saw not more than two cells (persisting megaspores) above the embryo-sac, the development should conform to the allium type. This statement is based on the assumption that the micropylar dyad cell undergoes division whereas the chalazal one develops directly into the embryo-sac. Long before Junell's (1934) publication, Treub (1883) mentioned the persisting megaspores as two " bubbles ' found around the free end of the embryo-sac. It is to be noted that neither Treub nor Junell actually followed the further development of the chalazal dyad cell. During the course of the present investigation, linear tetrads of megaspores were consistently observed. Therefore it may be categorically stated that in *Avicennia officinalis* the embryo-sac development follows the Polygonum type. It appears likely that a reinvestigation of *A. marina* may also indicate a normal type of embryo-sac development in this species.

The chalazal megaspore germinates into the functional eight-nucleate embryo-sac. The megaspore lying immediately above the chalazal one degenerates early and it is perhaps due to this fact that Junell observed only two cells capping the embryo-sac.

After degeneration of the nucellar epidermis the female gametophyte lies directly against the integumentary tapetum in *Lippia nodiflora*, *Vitex* and *Tectona* (Pal, 1951) whereas in *Avicennia* such modifications of the inner epidermal layers of the integument is wanting.

As a rule, the antipodals in Verbenaceæ degenerate quite early. Junell (1934) working on a number of species discovered that the antipodals in some species persist for a long time and show an increase in activity. In certain species the number of nuclei increases in each of the antipodal cells while in others there is an actual division of the antipodal cells themselves. Misra (1937) found as many as twenty antipodal cells in *Clerodendron phlomidis*. In *Lantana* (Tatachar, 1940; Padmanabhan, 1959) the number of nuclei in the antipodals increases up to eight. Tatachar believed that these multinucleate antipodals persist and act as the chalazal haustorium. However,

recently it has been shown that even though the antipodals in *Lantana* persist sometime after fertilization, degenerate without assuming any haustorial function (Padmanabhan, 1959).

Summary

The sequence of development of floral envelopes in ontogeny of *Avicennia officinalis* is as follows: calyx, androecium, corolla, gynœcium.

The sepals are vascularised by one median and two lateral strands. The petals and stamens receive one bundle each. The gynœcial wall is supplied by two large bundles and numerous smaller bundles from the stele of the floral axis. The placental column is vascularised by two median bundles (situated in a plane at right angles to that of the dorsal bundles) and four procambial strands. The median bundles fade out at the subterminal levels of the column, whereas the four procambial strands fuse in pairs and undergo torsion and split higher up into four traces that supply the ovules.

The young anther shows four groups of hypodermal archesporial cells in a cross-section. The archesporium cuts off a parietal layer which by successive periclinal divisions gives rise to four or five wall layers, the innermost of which differentiates into the tapetum while the outermost forms the endothecium. The tapetal cells are binucleate and are of the glandular type.

The microspore mother cell undergoes meiosis followed by cytokinesis in a simultaneous method. The microspore tetrads are usually tetrahedral; frequently decussate and isobilateral tetrads are also encountered. The pollen grains are shed at the two-celled stage.

The ovule is tenuinucellate and its integument shows somewhat arrested development as a consequence of which a conventional type of micropyle is not organised. The archesporial cell functions directly as the megaspore mother cell and after meiosis a linear tetrad of megaspores is formed, in contrast to the previous accounts that described or assumed a bisporic development of the female gametophyte. The chalazal megaspore germinates into the eight-nucleate mature embryo-sac while the megaspore situated immediately above it degenerates precociously. The two micropylar megaspores persist until fertilization. The antipodals are ephemeral. The egg-apparatus lies in the free micropylar end of the embryo-sac which protrudes into the locule.

Acknowledgement

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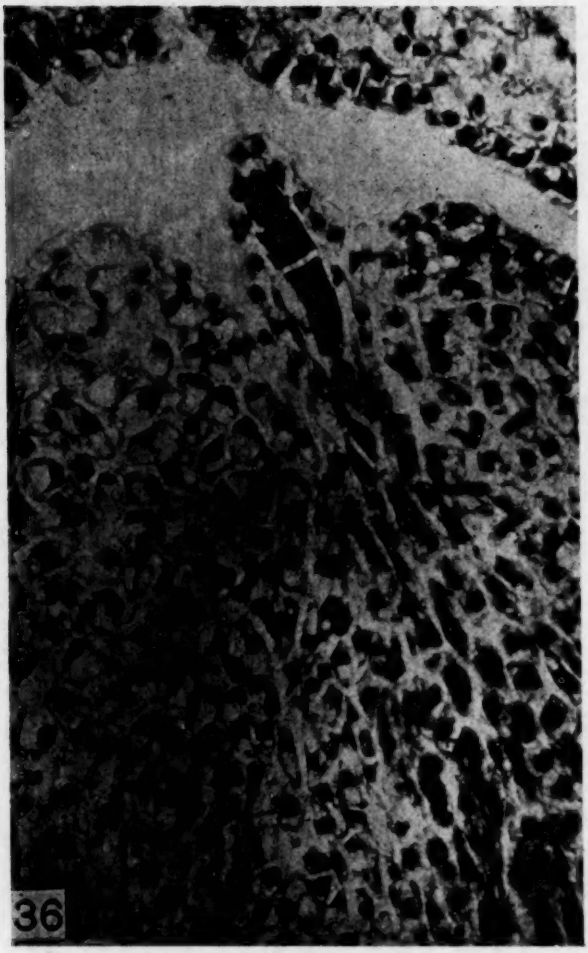

FIG. 36

Photograph of longisection through the micropylar end of the ovule showing nucellus with a linear tetrad of megaspores, ×425.

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STUDIES ON THE LEPTOCEPHALI OF BOMBAY WATERS

I. The Metamorphosing Stages of *Muræsox talabonoides* (Bleeker)*

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Introduction

The leptocephali of Indian waters have received very little attention till a decade back when the first attempt to metamorphose them in the laboratory was successfully accomplished (Nair, 1946 *a*, 1947). Since then the systematic study of the leptocephali of the coastal waters has received increasing attention in spite of the difficulties encountered in referring them to their adults and a few important papers have appeared in recent years. The leptocephalus of *Uroconger lepturus*, another common eel of the Madras coast, was identified by the myotome and vertebral counts taken in conjunction with the other distinctive characters shown by the larva and the adult (Nair, 1946 *b*). The occurrence of this leptocephalus along with those of *Congrellus anago*, *Muræsox cinereus* and *Muræna* sp. was recorded from the Gulf of Mannar (Nair, 1948). This was followed by an account of 3 types of eel eggs and 2 types of preleptocephaline larval stages collected from the same place (Nair and Bhimachar, 1950). Gopinath (1950) described 6 different leptocephali including those of *C. anago* described by him earlier (1946) and *M. cinereus* (Nair, 1947). Since the identification of the other 4 types of leptocephali in his collection was not possible, he described them as Leptocephali A, C, E and F and pointed out that the first 2 leptocephali belong to the ophichthyid group. Jones and Pantulu (1952) gave an account of the metamorphosing stages of *Muræsox talabon* and 4 murænid leptocephali collected from the Burhabulong and Hooghly estuaries in Orissa and Bengal respectively and doubtfully assigned one of them to *Muræna tile*. Later, these authors (Pantulu and Jones, 1954) described 3 varieties of murænid leptocephali collected from the Burhabulong estuary

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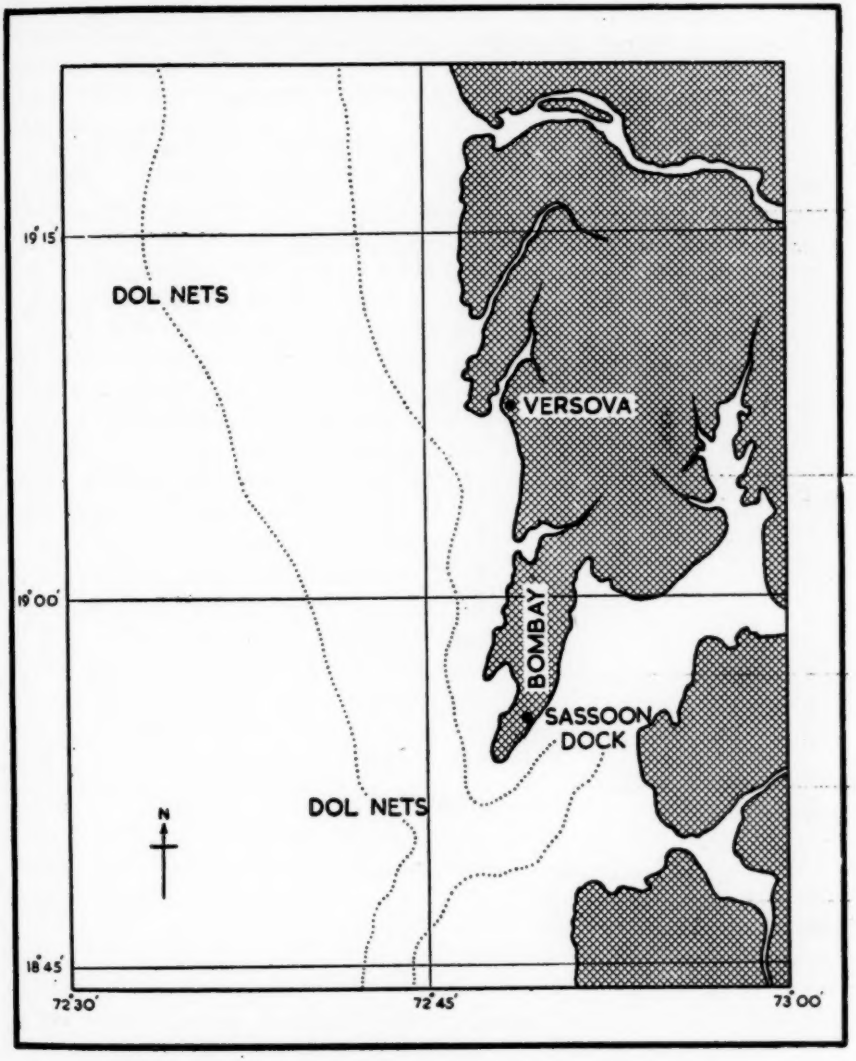
in Orissa. One of these larvæ was referred by them provisionally to *M. tile* and its metamorphosing stages were described by them. Recently, these authors (Jones and Pantulu, 1955) gave an account of 3 preleptocephaline larval stages of an ophichthyid eel together with descriptions of 4 ophichthyid leptocephali collected from Madras, Travancore and Orissa coasts and one was identified as that of *Pisoodonophis hijala*. Bapat (1955) while dealing with the eggs and larvæ of the Gulf of Mannar and the Palk Bay described an eel egg resembling Type II recorded previously from the former place by Nair and Bhimachar (1950). Recently Nair and Dharmamba (1960) described a few stages in the development of an ophichthyid egg collected from Lawson's Bay, Waltair.

The papers in this series dealing with the Bombay leptocephali were written by the senior author based on his study of a collection of larvae made by the junior author. These papers deal with the metamorphosis of *Muræsox talabonoides*, *Muræsox talabon* and *Uroconger lepturus* and descriptions of 5 leptocephali numbered serially I to V. Of these, Leptocephali I to III belong to Ophichthyidæ and Leptocephalus IV to Murænidæ while Leptocephalus V has not been identified.

MATERIAL AND METHODS

The material for these studies was collected from the 'dol' net fish catches landed at Versova and Sassoon Dock (*vide* Map) during the years 1952–56 with the exception of Leptocephalus III which was obtained from a plankton collection made at a distance of 20 miles west of Bombay on the 1st May 1953. Most of the leptocephali and elvers in the collection were obtained during the period April to June when shrimps dominated and formed more than 90% of the 'dol' net catches. The 'dol' nets are stationary nets which are fixed at a distance of about 10–15 miles off Bombay where the depth is about 10–15 fathoms. It is a long conical net the mouth of which is fastened to 2 huge wooden spikes fixed to the floor of the sea and is kept open by floats and ropes. The mesh size is greater near the mouth and diminishes towards the end and it varies from 10″ to less than ¼″. The net is worked entirely by the tidal current and it is reversed during the high and low tides. The net is, therefore, not selective in its action but traps all varieties of fish carried by the currents. The leptocephali picked out from these catches were fixed in 5% formalin in the field itself and sorted out later in the laboratory. Table I gives the place of collection, dates and number of larvæ and elvers collected from the 'dol' net catches.

The different stages, representing the growth and metamorphosis of the leptocephali dealt with in these papers, were selected arbitrarily to show the



Map showing the 'dol' net regions from where the material was collected.

important changes undergone during the transformation and an attempt has been made to divide the metamorphosis into 6 stages as proposed by Schmidt (1906) and Jespersen (1942). However, this was possible only in

TABLE I

Details of collection of Bombay leptocephali

Species	Date	Place	Number of lepto-cephalus	Number of elver
Muraenesox talabonoides	14- 4-1952	Sassoon Dock	2	9
	15- 4-1952	,,	1	2
	29- 4-1953	,,	1	2
	1- 5-1953	,,	6	..
	21- 4-1954	,,	32	4
	22- 4-1954	,,	11	10
	23- 4-1954	,,	37	2
	20- 5-1954	,,	..	44
Muraenesox talabon ..	1- 5-1953	Sassoon Dock	5	..
	21- 4-1954	,,	6	..
	23- 4-1954	,,	13	..
	15- 9-1954	,,	6	2
	5- 3-1955	,,	2	..
	9- 1-1956	Versova	1	..
Uroconger lepturus ..	9- 1-1954	Versova	48	13
	15- 9-1954	Sassoon Dock	3	7
	7-11-1954	Versova	3	..
Leptocephalus I ..	13- 9-1955	Versova	13	..
	24- 9-1955	Sassoon Dock	1	..
	9- 1-1956	Versova	29	..
Leptocephalus II ..	13- 9-1955	Versova	13	..
	9- 1-1956	,,	1	..
Leptocephalus III ..	1- 5-1953	Plankton off Bombay	1	..
Leptocephalus IV ..	9- 1-1956	Versova	1	..
Leptocephalus V ..	21- 4-1954	Sassoon Dock	1	..

the case of *Muræsox talabonoides* while in the others, the lack of representative and suitable specimens made it necessary to reduce the number of metamorphosing stages. All the growing and metamorphosing stages described in these papers are denoted with letters and numbers respectively.

In these studies emphasis has been given to the myotome number which is one of the chief characters for referring the leptocephali to their parent forms. The counting of the myotomes in all the larvæ was very simple but in *Uroconger lepturus* the caudal portion presented some difficulty and this was overcome by staining with eosin. While counting the preanal myotomes, if the vent happened to be below a particular myotome, this was also included under the preanal myotome number. In the descriptive accounts, due stress has been given to the morphometric characters and pigmentation which are found to be very helpful in the identification of the different larvæ and metamorphosing and elver stages. All the measurements given are in millimeter and they are also expressed as percentages of total length and length of head. The diameter of the eye and the length of snout are given as percentages of length of head while all the other measurements are given as percentages of total length and these percentages are given within brackets in the different tables. The dentition has also been studied in detail and the lower jaw in most cases presented some difficulty in counting the teeth present on it since its hind region is hidden by the upper jaw and this was overcome by keeping the mouth of the larva wide open with a small wedge-shaped glass bit.

A few abbreviations are used in the descriptive accounts and their full explanations are given below:—

Length	..	Total length from the tip of the snout to the end of the caudal fin.
Height	..	Maximum height including the vertical fins.
Head	..	Length of head from the tip of the snout to the gill opening.
Eye	..	Maximum diameter of the eye.
Predorsal distance	..	Distance between the tip of the snout and the origin of the dorsal fin.
Preanal distance	..	Distance between the tip of the snout and the anus.

I. The Metamorphosing Stages of *Muræsox talabonoides* (Bleeker)

Like the adult which mainly constitutes the eel fishery of the Bombay coast, the leptocephalus is very common in the Bombay

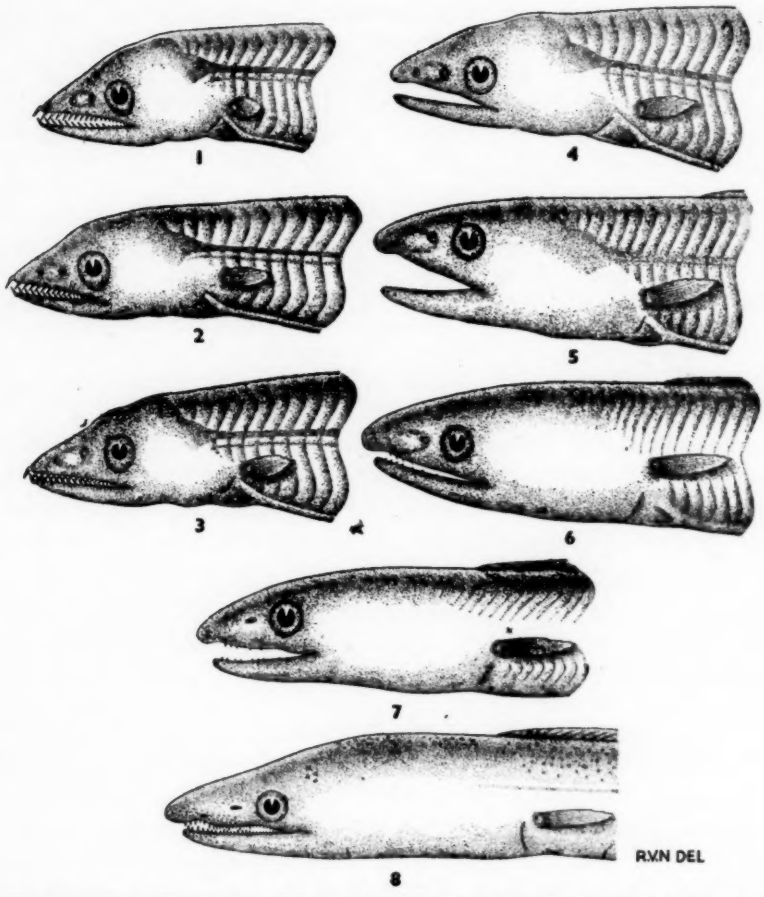
waters and all the larvæ and elvers in the collection were obtaine from the 'dol' net catches landed at Sassoon Dock only. They generally occur during the months of April and May and the larvæ are more abundant during the month of April. The majority of the specimens were in advanced stages of metamorphosis and out of the several specimens in the collection only 9 larvæ, including the growing stages, showed the complete set of larval teeth. The myotome variation found in the species is very low and the number ranged from 147 to 149 only.

Stage A (Text-Fig. 1).—The growing stages are extremely rare and only 2 specimens were collected on 1st May 1953 along with the metamorphosing stages and the smaller of the two is selected as the type of this stage.

The larva is short measuring 56·5 mm. in length, moderately thick and tapers conspicuously towards the head and the tail regions showing a characteristic spindle-shaped appearance. The maximum height is observed in the middle portion and it measures 5·5 mm. and is 10·3 times in length.

The head is fairly long and measures 4·5 mm. and is contained 12·6 times in length. The snout is moderately long, sharply pointed and is 3 times in head. The nostrils have differentiated and the anterior one is situated midway between the tip of the snout and the front border of the eye while the posterior one is in the middle of the interspace between the anterior nostril and the eye. The cleft of the mouth is nearly straight and extends up to the posterior border of the eye. Both the jaws are of about the same length and are provided with well-developed teeth which are directed forwards. The dental formula is $1 + 10 + 8 / 1 + 10 + 6$. Each half of the upper jaw is provided with a long, curved and pointed grasping tooth and two groups of teeth of unequal size. The first group is composed of 10 teeth which are also long and pointed and show gradual decrease in length backwards. The second set is composed of 8 short and conical teeth which are compactly arranged in the posterior third of the upper jaw. The lower jaw carries in each half a grasping tooth at its tip followed by two groups of teeth consisting of 10 and 6 teeth respectively. The teeth of these two groups are similar to those of the upper jaw in shape, size and arrangement. The eye is egg-shaped with the pointed portion directed downwards and it is 5 times in head.

The myotomes are distinctly seen and number 148 of which 92 are preanal in position. The alimentary canal is very long, straight and occupies nearly $\frac{3}{4}$ the length of the larva with the preanal distance 1·4 in length. The dorsal fin is long and its origin is above the 30th myotome. The predorsal distance is very short and it is 3·8 times in length.



TEXT-FIGS. 1–8. Head region of the growing and metamorphosing stages of the leptocephalus of *Muræsox talabonoides*, ×*ca.* 5·5.

The pectoral fin is very small and shows only indistinct rays. The vertical fins are well developed and possess distinct rays which show only slight variation in number in the different specimens. In the type specimen there are about 254 and 205 moderately sized rays in the dorsal and anal fins respectively. The caudal fin shows 8 distinct rays which are very long and about 4 times the length of the rays of the other vertical fins. The long caudal fin rays give a pointed appearance to the tail.

The pigmentation of the larva is feeble and the chromatophores are present in moderate numbers only. However, their arrangement shows a definite pattern which is quite distinct from that found in the leptocephalus of *Muræsox cinereus* (Nair, 1947) and *Muræsox talabon* (Nair and Mohamed, 1960), the other two species of the genus recorded from the Indian waters. This characteristic colouration has been noticed in all the larval, metamorphosing and elver stages and as such it has considerable taxonomic importance in the identification of the larva of this species.

The head pigmentation is feeble and consists of 2 or 3 black pigment cells arranged along the margin of the middle portion of the upper jaw and 1 or 2 similar larger chromatophores in the heart region. The tip of the lower jaw is also provided with 1 or 2 highly branching black pigment cells.

The mid-lateral row of pigment cells commences from the 23rd myotome and the chromatophores are arranged in a line below the vertebral column. The pigment cells of this row are large, stellate and show an irregular distribution at intervals of 2–4 and even 5 myotomes. In the anterior region they are generally distributed at intervals of 3–4 myotomes while posteriorly they are arranged closer and usually every alternate myotome is provided with a chromatophore.

The regularity in the disposition of the chromatophores of the alimentary canal gives the larva a characteristic colouration. The pigment cells are arranged in groups at regular intervals on the ventral side of the alimentary canal in the form of conspicuous dark patches (Photograph 2). Generally 6–8 and occasionally 9 such pigment groups are seen and each group is composed of 4–25 chromatophores. In all the specimens, these groups occupy more or less the same position in relation to the myotomes, especially the first 6 groups which are situated in the aortic portion of the alimentary canal. The last two groups found in the post-aortic portion do not generally occupy the same position in the different larvæ since this region shows variations in length depending on the stage of growth of the larva. It is also seen that these two groups, especially the last one situated near the anus, become unrecognisable in the metamorphosing stages owing to the rearrangement of the chromatophores. In the type larva described here 8 pigment groups are present and the 6 aortic groups are found under myotomes 12–13, 20–21, 27–28, 33–34, 41–42 and 48–49. Of these, the second group situated below the liver diverticulum is large, conspicuous and composed of a large number of chromatophores while the others are relatively smaller with lesser number of pigment cells. The seventh group, which is the first post-aortic group, is not conspicuous in the type specimen owing to the diffuse arrangement of

the pigment cells below myotomes 70–74. The last group is situated in the terminal portion of the alimentary canal. In between these groups stray black chromatophores have also been noticed.

The pectoral and dorsal fins are unpigmented while a few scattered pigment cells are present at the base of the anal fin. Amidst the rays of this fin highly elongated chromatophores are found at irregular interval throughout its length. A few black branching chromatophores are present in the caudal fin.

Stage B (Text-Fig. 2).—This growing stage, like the preceding one, is extremely rare in the Bombay waters and only 3 specimens are present in the collection. This larva represents an intermediate stage between Stage A and the full-grown leptocephalus described under Stage I.

The spindle-shaped appearance is lost in this stage and the larva is moderately long and measures 73·5 mm. The height has increased proportionately and it is 5·8 mm. which is 12·7 times in length.

The head has elongated slightly and measures 4·9 mm. and it is 15 times in length. The snout is long and pointed and it is 2·9 times in head. The nostrils are seen distinctly and the anterior one is situated at a third of the distance from the tip of the snout to the eye while the posterior one is midway between the front margin of the eye and the anterior nostril. No change in the shape of the eye is observed in this stage and it is 4·9 times in head. There is no increase in the number of teeth, but the teeth formula is $1 + 10 + 8 / 1 + 16$ owing to the slight increase in the size of the teeth composing the second group of the lower jaw as a result of which the differentiation into two groups is no longer seen and all of them show a uniform reduction in length backwards.

The origin of the dorsal fin has shifted forwards and it is above the 28th myotome and the proportion of the predorsal distance in length has increased to 4·7 due to the lengthening of the larva. The alimentary canal does not show any change except in the shifting of the anus which is below the 90th myotome. There is only a slight reduction in the preanal distance which continues to be 1·4 times in length.

The pectoral fin has grown larger and it is long and pointed with indistinct rays. The vertical fins do not show any change and the dorsal and anal fins in the type specimen show 269 and 208 rays respectively. The caudal fin rays, which number 9 in all the specimens, have elongated considerably.

There is no change in the pigmentation of the larva and it generally conforms with that of the previous stage. The alimentary canal of the type specimen, described here, shows 9 pigment groups and the additional group is formed by the breaking up of the third pigment group into two distinct ones located below myotomes 25 and 28–29. Of these two groups, the first one is smaller in size while the second one is of the same size as the others in the series. The other five aortic groups of chromatophores are situated under myotomes 12, 18–19, 33–34, 40 and 46–47 while the two post-aortic groups show variation in their position in the different specimens and in the type specimen they are under myotomes 68–69 and 80–81. Unlike the condition found in the preceding stage, the last group is placed slightly in front of the vent while a few scattered pigment cells are present near the anal opening.

Stage I (Text-Fig. 3 and Photograph 1).—Full-grown leptocephalus with all the larval teeth intact is extremely rare and is represented in the collection by a few specimens only and they are more or less similar in the morphometric characters and show only slight variations in size and in the position of the anal opening.

The leptocephalus is thin and long with a pointed tail and it measures 81·0 mm. in length. The maximum height of 6·2 mm. is observed in the middle region and it is 13·1 times in length.

The head measures 5·3 mm. and it is 15·3 times in length. During the growth of the larva into the leptocephalus, the increase in the length of the head is not porportionate to that of the length but is somewhat slow as seen clearly from Table II which gives the morphometric characters of the different stages described here. The snout is long and pointed and it is 2·8 times in head. Unlike the head, the snout shows higher increase in length in all the growing stages. The eye is somewhat oval in shape and does not show any increase in size and it is 5·9 times in head. The nostrils are distinctly seen as small apertures and the posterior nostril is in front of the eye while the anterior one is midway between the tip of the snout and the posterior nostril. The cleft of the mouth continues to be straight and extends up to the hind border of the eye. Minor changes in the number of teeth are noticed in this stage and in some specimens the grasping tooth of the upper jaw is not seen and this is believed to be lost accidentally. A few specimens showed the full set of teeth in both the jaws and the dental formula in the full-grown leptocephalus is $1 + 10 + 9 / 1 + 16$. It is seen that one more tooth is added to the second group of the upper jaw thus increasing the total number in each half of the jaw to 20 teeth which do not show any change in size or arrangement.

TABLE II

Measurements of Murænesox talabonoides

	Stage A	Stage B	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI
Total length ..	56·5	73·5	81·0	85·0	83·5	78·0	77·5	78·5
Maximum height ..	5·5 (9·7)	5·8 (7·9)	6·2 (7·7)	5·9 (6·9)	5·7 (6·8)	4·8 (6·2)	3·3 (4·3)	3·0 (3·8)
Predorsal distance ..	14·8 (26·2)	15·8 (21·5)	13·8 (17·0)	10·8 (12·7)	8·8 (10·5)	8·1 (10·4)	7·8 (10·1)	9·3 (11·8)
Preanal distance ..	40·5 (71·7)	51·5 (70·1)	46·2 (57·0)	38·1 (44·8)	31·3 (37·5)	28·0 (35·9)	27·0 (34·8)	26·5 (33·8)
Length of head ..	4·5 (8·0)	4·9 (6·7)	5·3 (6·5)	5·7 (6·7)	7·3 (8·7)	7·6 (9·7)	7·8 (10·1)	10·0 (12·7)
Length of snout ..	1·5 (33·3)	1·7 (34·7)	1·9 (35·8)	1·9 (33·3)	2·0 (27·4)	2·1 (27·6)	2·1 (26·9)	2·5 (25·0)
Diameter of eye ..	0·9 (20·0)	1·0 (20·4)	0·9 (17·0)	0·9 (15·8)	1·0 (13·7)	0·9 (11·8)	0·9 (11·5)	0·9 (9·0)
Total myotomes ..	148	148	149	148	149	149	146*	146*
Preanal myotomes ..	92	90	74	58	51	46	44*	42*
Predorsal myotomes ..	30	28	21	16	13	11	8*	8*

* Vertebræ.

In the type specimen 149 myotomes are present and the vent is situated below the 74th myotome. The anterior position of the vent shows that the anal migration has commenced even before the onset of metamorphosis which is generally indicated by the shedding of the larval teeth. Further, the highest preanal myotome number is reached in this species during the growing stage itself and its reduction in the full-grown leptocephalus makes this character of doubtful taxonomic importance in the identification of the larva. The most backward position of the anus is seen in Stage A where it is below the 92nd myotome while in Stage B it is below the 90th myotome. In the full-grown leptocephalus the anus occupies a far anterior position, below the 74th myotome, showing considerable reduction in the number of preanal myotomes and also the length of the alimentary canal indicating clearly that anal migration precedes metamorphosis. The origin of the dorsal fin also shows a forward migration and it is above the 21st myotome in this stage. This character also, like the preanal myotome number, is not useful for the identification of the leptocephalus of this eel. The predorsal distance is contained 5·9 times in length. The anal opening is slightly behind the middle of the larva and the preanal distance is 1·8 times in length.

The pectoral fin is long and pointed with distinct rays. The dorsal and anal fins show 267 and 205 rays respectively while the caudal fin in the type specimen possesses 10 distinct rays.

The pigmentation of the full-grown leptocephalus is moderate when compared with that of the larvæ of the other two species of *Muræsox*. The leptocephalus does not show any significant change in the pigmentation from that of the growing stages. In this type specimen also the alimentary canal shows 9 groups of chromatophores of which 7 are located below the aorta while the remaining 2 are behind it. These groups are found below myotomes 11–12, 20, 25, 29, 33, 40–41, 47, 62–63 and 69–70. The additional group observed in this specimen is situated below the 29th myotome. The second post-aortic pigment group is near the anal opening.

Stage II (Text-Fig. 4 and Photograph 1).—This is the edentulous stage indicating the commencement of metamorphosis and the only noteworthy difference from Stage I is the complete shedding of all the larval teeth.

A very slight reduction in height, which measures 5·9 mm., has taken place and it is 14·4 times in length. The head measures 5·7 mm. which is 14·9 times in length. The snout is 3 times in head and in this respect resembles the condition found in Stage A. The eye is oval in shape and it is 6·3 times in head.

The myotomes are distinct and their number in the type specimen is 148. There is considerable reduction in the length of the alimentary canal and the vent, which is below the 58th myotome, is slightly in front of the middle with the preanal distance 2·2 times in length. The origin of the dorsal fin has also shifted anteriorly and it is above the 16th myotome, with a proportionate reduction in the predorsal distance which is 7·9 times in length.

The paired and vertical fins do not show any change in this stage.

There is no major change in the pigmentation except the slight variation brought about by the reduction in the length of the alimentary canal. Only 6 pigment groups are seen in this stage and they are situated below the aorta under myotomes 13, 18–19, 26–27, 32–33, 38 and 45–46. In this stage the alimentary canal is slightly shorter than the aorta and consequently the chromatophores of the 2 post-aortic groups have become scattered and are found near the end of the gut and some of them are in a circle round the anal opening.

Stage III (Text-Fig. 5 and Photograph 1).—This metamorphosing stage is somewhat opaque due to the reduction in height and increase in thickness. The caudal portion of the larva tapers conspicuously and ends in the pointed tail. The maximum height of 5·7 mm. is at the region of the anus and the height is 14·6 times in length.

The head has elongated and is somewhat cylindrical in shape. The length of the head is 7·3 mm. which is 11·4 times in length. The snout, on the other hand, does not show proportionate increase and it is 3·7 times in head. It is bluntly rounded with a constriction at its tip and projects beyond the lower jaw. The eye in this stage is somewhat circular and it is 7·3 times in head.

Both the vent and the origin of the dorsal fin show further anterior shifting and they are situated opposite myotomes 51 and 13 respectively. The origin of the dorsal is in advance of the tip of the pectoral fin with a very short predorsal distance which is 9·5 times in length. The vent is situated at about the anterior third of the larva and the preanal distance is 2·7 times in length.

The pectoral fin has become slightly longer and the vertical fins are better developed and more prominent in this stage. The caudal fin is provided with 10 rays which have become elongated.

Pigmentation remains unchanged and due to the opacity of the metamorphosing larva, the chromatophores in the heart region are not clearly visible while the body pigmentation is seen distinctly. The six prominent

groups on the alimentary canal are seen opposite the myotomes 13, 19, 27–28, 34, 41 and 48. The other groups are not distinct and their chromatophores are found on either side of the anal opening.

Stage IV (Text-Fig. 6 and Photograph 1).—In this stage the metamorphosing larva has become almost opaque due to the thickening of the body. There is also great reduction in the height which now measures only 4·8 mm. and it is 16·3 times in length.

The head has elongated and its general shape very nearly approaches that of the adult head. The lengthening of the head is conspicuous in the post-orbital region while the snout shows only very slight increase in length. The head measures 7·6 mm. which is 10·3 times in length while the snout is 3·6 times in head. The upper jaw is longer and projects beyond the lower and shows the rostral constriction clearly. Several minute, conical and backwardly directed teeth, representing the adult set of teeth, have appeared as small protuberances in both the jaws. There is no change in the position of the nostrils except in the slight shifting of the anterior one towards the border of the upper jaw. The eye also does not show any change in size or in shape and it is 8·4 times in head.

The myotomes in this stage could be made out only with some difficulty owing to the opacity of the larva. However, reflected light was found to be helpful in counting their number and the type specimen showed 149 myotomes. The anus has shifted further anteriorly and it is below the 46th myotome. The alimentary canal is short and the preanal distance is contained 2·8 times in length. The origin of the dorsal fin also shows a forward shifting and it is above the 11th myotome and in a line with the base of the pectoral fin. There is slight reduction in the predorsal distance and it is 9·6 times in length.

The fins do not show any change except for the slight increase in the length of the pectoral and caudal fins.

The pigmentation shows some variation from that of the preceding stage. A few small brown pigment cells have appeared at the tip of the snout. The body pigmentation is fairly prominent, particularly along the mid-lateral line and the alimentary canal. Some of the pigment cells of the mid-lateral series and of the caudal portion have become elongated and are arranged along the myocommas. The reduction in the length of the gut has resulted in the further fusion of the posterior groups of pigment cells of the alimentary canal and in this stage the 5 anteriormost ones alone are seen distinctly and they are situated opposite myotomes 12, 18, 26, 35 and 40. The chromatophores of these groups have become compactly arranged rendering them very

conspicuous in this stage. Due to the shifting of the anus the chromatophores of the succeeding groups have merged to form a single group which is in the form of an irregular short streak on either side of the terminal portion of the alimentary canal and the anal opening, very much like the condition found in *Muræsox talabon* (Nair and Mohamed, 1960). Several minute chromatophores have appeared along the periphery of the middle portion of the dorsal fin while its extremities are practically devoid of pigment cells. In addition to the chromatophores in the caudal fin, a few more black pigment cells have appeared along its fin rays.

Stage V (Text-Fig. 7 and Photograph 1).—In this stage the metamorphosing larva has become completely opaque, the leptocephaline shape is almost lost and the cylindrical shape of the elver is assumed. The height is more or less uniform in the anterior region while posteriorly it decreases markedly. The height is 3·3 mm. which is 23·5 times in length.

The head has also undergone changes in shape and resembles the adult head excepting in colouration. The length of the head has increased to 7·8 mm. which is 9·9 times in length. The snout does not show any appreciable change in length and it is 3·7 times in head. It is pointed and its tip projects far beyond that of the lower jaw. The rostral constriction is conspicuously seen in this stage. The anterior nostril has become slit-like while the posterior one appears as an oval aperture and both of them do not show any change in their relative positions. The gape of the mouth is longer in this stage and extends behind the eye by about half its diameter. The teeth have grown slightly longer and they are distinctly seen as conical, pointed and incurved projections. The eye continues to be of the same shape and size and is contained 8·7 times in head.

The myotomes could not be made out in this stage and alizarin preparation showed 146 vertebræ excluding the hypural bones. The vent shows a further forward migration and it is below the 44th vertebra with the preanal distance 2·9 times in length. The origin of the dorsal fin has also shifted anteriorly and it is opposite the 8th vertebra. The origin of the dorsal is in advance of that of the pectoral fin and it is opposite the gill opening. The predorsal distance is 9·9 times in length.

The fins do not show any noticeable change except for the slight increase in the length of the pectoral and caudal fins.

A slight intensification of the general pigmentation is seen in this stage. Several minute brown chromatophores have appeared in the snout along the border of the upper jaw giving a light-brown colouration to this portion.

The mid-lateral row of pigment cells is seen very prominently. The pigmentation of the alimentary canal is also conspicuous and the 5 pigment groups present in this stage are located below vertebræ 12, 18, 25, 36 and 42. As in the previous stage, the chromatophores of the other groups have become compactly arranged to form two short prominent streaks on either side of the terminal portion of the gut as well as the anus. These 6 conspicuous congregations of black chromatophores give a characteristic spotted appearance to this stage. Several additional brown chromatophores have appeared along the border of both the dorsal and anal fins giving their edges a light-brown colouration especially in the posterior region.

Stage VI (Text-Fig. 8 and Photograph 1).—The elvers which represent the final phase in the transformation of the leptocephalus resemble the adult in every respect excepting in colouration. The assumption of the adult colouration is somewhat slow and is acquired only in the growing elvers. Voracious feeding is observed in this stage and most of the specimens in the collection showed their stomachs gorged with shrimps, *Acetes*, etc.

The body has become perfectly cylindrical with the height uniform in the anterior region while posteriorly it tapers imperceptibly into the pointed tail. The maximum height of the elver is only 3·0 mm. and it is 26·2 times in length which is about 2½ times the proportion found in the leptocephalus.

The head has become very long and measures 10·0 mm. and it is 7·9 times in length which is nearly twice the proportion seen in the larva. The shape of the head shows some changes in the orbital and post-orbital regions brought about by the reduction in its height. The snout is pointed and shows considerable increase in length, but not proportionate to that of the head and it is 4 times in head. The rostral constriction has become very distinct and conspicuous in the elver stage. The posterior nostril is in the form of a moderately long slit in front of the eye while the anterior one which is also slit-like is situated behind the notch of the snout about midway between the posterior nostril and the tip of the snout. It may be pointed out here that the anterior nostril in *Muræsox cinereus* becomes distinctly tubular even in the elver stage (Nair, 1947; Text-Fig. 6) while their tubulate nature is hardly made out even in the advanced elvers of this species. In the juveniles measuring over 20 cm. in length the tubular nature of the nostril is not seen distinctly. The eye is perfectly circular and it is 11·1 times in head. The cleft of the mouth of the elver is longer and extends behind the eye by about ¾ its diameter. The teeth which have become longer and stronger show differential growth and some of them have already become double the size of the others indicating that they ultimately form the canine teeth of the adult.

None of the myotomes could be made out in the elver and alizarin preparation showed the presence of 146 vertebræ excluding the hypural bones. The anus shows further forward shifting and it now occupies the adult position under the 42nd vertebra. The preanal distance is also reduced and it is 3 times in length. The origin of the dorsal fin in relation to the vertebra does not show any change and it continues to be above the 8th vertebra indicating that the adult position is reached in the previous stage itself. The origin of the dorsal fin is far in advance of that of the pectoral fin and also the gill opening and consequently the predorsal distance is shorter than the length of the head. However, this distance in the elver is longer than that of the previous stage owing to the lengthening of the head and it is 8·4 times in length.

The pectoral and caudal fins have become longer and more pointed and resemble those of *Muræsox talabon* (Nair and Mohamed, 1960). The lateral line is seen distinctly in the elver.

The elver shows certain changes in the general pigmentation which more or less conform with the pattern of colouration of the adult. The larval pigmentation is conspicuously seen in the elver and the retention of this colouration even in the advanced elver is highly helpful in its identification from those of the other two species. The brown pigmentation at the tips of the jaws and the ventral border of the snout has become prominent due to the addition of several minute chromatophores. The dorsal side of the snout and the interorbital region are free from chromatophores. A few scattered pigment cells have appeared in two groups on the dorsal side of the head in front of the dorsal fin.

On either side of the dorsal fin, several uniformly distributed brown dendritic pigment cells have appeared giving a faint-brown colouration to the dorsal side of the elver. These pigment cells are more numerous in the anterior region while they are somewhat diffusely scattered in the tail region with the caudal extremity free from chromatophores. The mid-lateral pigmentation, comprising of large, black pigment cells, is conspicuously seen as a row of dots distributed at equal intervals just below the lateral line. Some of the pigment cells in the tail region are linear in shape and are placed transversely. This row does not extend up to the tail of the elver.

The type elver shows 6 prominent pigment cell groups on the ventral side of the alimentary canal. Of these 6 groups, the first 5 constitute the original larval groups while the last one situated near the anus is a composite one formed by the fusion of the other larval groups of pigment cells. The chromatophores of this group encircle the anal opening of the elver.

The edges of both the anal and dorsal fins are brown in colour due to the appearance of numerous, finely branching, brown chromatophores which merge with one another to give a uniform colouration. These pigment cells are more abundant in the posterior region of these fins giving them a dark-brown colour. The anteriormost regions of both these vertical fins are without any chromatophores.

REMARKS

The foregoing account which deals with 2 growing stages and 6 metamorphosing stages of the larva of *Muraenesox talabonoides* shows clearly the changes undergone during the two phases in its life-history. The marked increase and decrease in the length of the larva noticed during the growing and metamorphosing phases respectively are seen clearly from Table II which gives the morphometric characters of all the different stages. The height shows uniform reduction in all the stages due to the increase in length of the growing stages and decrease in length and height of the metamorphosing stages. A similar reduction in the predorsal and preanal distances and the size of the eye is also noticed in these two phases. The head, on the other hand, shows gradual reduction in length during the growth of the larva while it progressively increases in the different metamorphosing stages. This is due to the differential rate of growth of the head and the body seen in the growing and metamorphosing stages. It is seen that the growing stages show a higher rate of lengthening of the body than that of the head while the metamorphosing stages show reduction in body length and the elongation of head length. On the contrary the snout length increases in the growing stages and decreases in the metamorphosing stages. It may be pointed out that the increase in the length of the snout is higher than that of the head during the growing period while the opposite condition is seen during the metamorphosing period. As a result of the disproportionate growth during the two phases, the shortest head length and the longest snout length are seen only in the full-grown leptocephalus.

Another interesting feature observed during the growing period is the anterior migration of both the anus and the origin of the dorsal fin which takes place even before the leptocephalus reaches its full growth. This phenomenon has been observed in a few other Indian leptocephali as well and in this respect some of the Indian leptocephali differ from those of the temperate waters where the migration of the anus takes place only after the full growth of the larva is reached. It is likely that this acceleration in the metamorphosis is caused by the higher temperature conditions of the tropical waters.

The leptocephali of *Muræsox talabonoides* and *Muræsox cinereus* (Nair, 1947) differ markedly both in external and morphometric characters (Tables II and III). The most striking difference is in the height which in *M. cinereus* is considerably greater giving it the typical leaf-like appearance. The height of *M. talabonoides* is only half that of *M. cinereus* and consequently presents a linear and elongated appearance. In the full-grown leptocephalus of *M. talabonoides*, both the predorsal and preanal distances are shorter than those of *M. cinereus*. The head in these two species shows only slight difference in length. However, the snout in *M. talabonoides* is shorter than that of *M. cinereus* while the eye in the former is larger than that of the latter. The total myotome number in *M. talabonoides* is 149 which is higher than that of *M. cinereus* with a difference of 11 myotomes. Similarly, the vent in the full-grown larva of *M. talabonoides*, even though not comparable in view of its shifting in the growing stages itself shows slight difference in position from the condition found in *M. cinereus*. These two species differ in the number of teeth also and *M. talabonoides* shows 20/17 while *M. cinereus* possesses a lower number of 15/15 teeth only. The shape of the pectoral fin also aids in the identification and it is long and pointed in *M. talabonoides* while it is circular in *M. cinereus*.

Apart from these differences in general shape and body proportions, the pigmentation of the two larvæ shows striking differences. There is close similarity in the arrangement of the pigment cells in the head region of these two species. It may, however, be pointed out that the tip of the lower jaw is provided with a few chromatophores in *M. talabonoides* while such pigment cells are not present in *M. cinereus*. The arrangement of the mid-lateral chromatophores in the two species is also helpful in the identification and the number of pigment cells is much less in *M. talabonoides* since they begin from the 23rd myotome only and are widely spaced while in *M. cinereus* the row commences from the 17th myotome and all the succeeding myotomes are provided with pigment cells. The most important difference is found in the grouping of the chromatophores along the alimentary canal in *M. talabonoides* in the form of 6–9 dark patches while they are distributed along the entire length of the alimentary canal in *M. cinereus* without any regularity in their arrangement (Photograph 3). This character, as stated earlier, was successfully used in the identification of not only the leptocephalus, but the metamorphosing stages and even the advanced elvers.

The metamorphosing stages of the leptocephalus of *M. talabonoides* show the gradual assumption of the adult characters and the pattern of changes seen in this species generally tallies with that found in *M. cinereus*.

TABLE III

Measurements of Muræsox cinereus (*Nair*, 1947)

	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI
Total length ..	81·0	73·0	67·0	68·0	61·0	62·0
Maximum height (including fins) ..	11·0 (13·6)	8·0 (11·0)	7·5 (11·2)	7·0 (10·3)	6·0 (9·8)	4·5 (7·3)
Maximum height (excluding fins) ..	10·0 (12·3)	6·5 (8·9)	5·0 (7·5)	4·5 (6·6)	3·0 (4·9)	2·8 (4·5)
Predorsal distance ..	20·0 (24·7)	11·5 (15·8)	10·0 (14·9)	9·0 (13·2)	7·5 (12·3)	8·5 (13·7)
Preanal distance ..	53·0 (65·4)	31·0 (42·5)	25·0 (37·3)	27·0 (39·7)	21·0 (34·4)	21·0 (33·9)
Length of head ..	5·0 (6·2)	6·0 (8·2)	6·5 (9·7)	6·5 (9·6)	7·0 (11·5)	8·5 (13·7)
Length of snout* ..	2·2 (44·0)	2·3 (38·3)	2·1 (32·3)	2·2 (33·8)	2·1 (30·0)	2·6 (30·6)
Diameter of eye* ..	0·7 (14·0)	0·8 (13·3)	0·8 (12·3)	0·9 (13·8)	0·7 (10·0)	0·9 (10·6)
Total myotomes ..	138	..	..	..	..	..
Preanal myotomes ..	78	50	44	43	..	..

* Calculated from the Text-Figures.

However, in *M. talabonoides* indications of the adult colouration are laid in the elver stage only while in *M. cinereus* they are clearly noticeable on the body even during the metamorphosing stages and the pigmentation of the elver generally resembles that of the adult. The slow appearance of the adult colouration in *M. talabonoides* is due to the fact that this species is somewhat lighter in colour and grows to a larger size than *M. cinereus*. The anterior nostril in *M. cinereus* becomes distinctly tubular in the elver stage while its tubular nature is not even indicated in the elver of *M. talabonoides* and the advanced elvers present in the collection do not also show this character distinctly.

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EXPLANATION OF PLATE

PHOTOGRAPH 1. The six stages in the metamorphosis of *Muraenesox talabonoides*. About natural size.

PHOTOGRAPH 2. A group of pigment cells on the alimentary canal of *Muraenesox talabonoides*.

PHOTOGRAPH 3. The scattered pigment cells on the alimentary canal of *Muraenesox cinereus*.

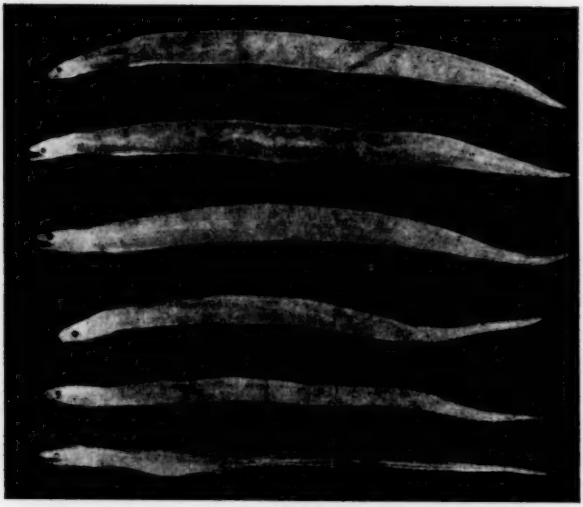

PHOTO. 1

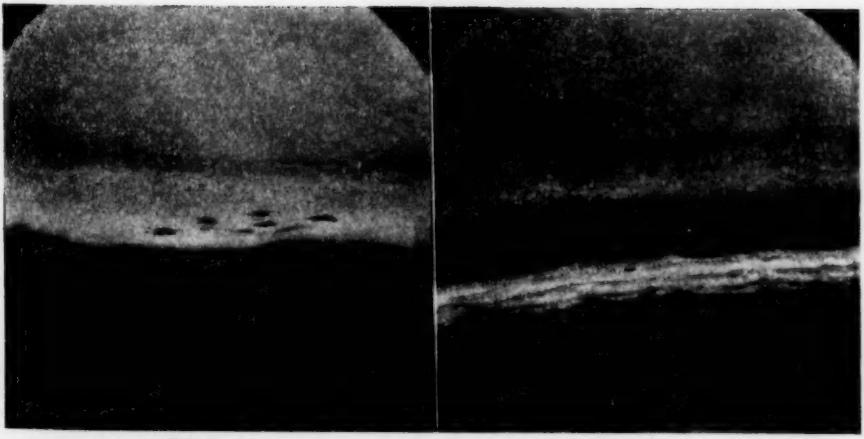

PHOTO. 2

PHOTO. 3

STUDIES ON THE LEPTOCEPHALI OF BOMBAY WATERS

II. The Metamorphosing Stages of *Muræsox talabon* (Cantor)*

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The leptocephalus of *Muræsox talabon* generally occurs in the 'dol' net catches along with that of *Muræsox talabonoides* and the collection contains several specimens and 2 elvers (*vide* Introduction, Nair and Mohamed, 1960 *a*). Some of the larvæ showed the full set of larval teeth while in the others they have fallen off indicating the commencement of metamorphosis. A few leptocephali showing intermediate stages of metamorphosis and the elvers present in the collection made it possible to get a more or less connected series of stages in the transformation. It may be pointed out that Stage V described here represents the elver stage and corresponds to Stage VI in the metamorphosis of *Muræsox cinereus* (Nair, 1947) and *Muræsox talabonoides* (Nair and Mohamed, 1960 *a*). The penultimate stage, corresponding to Stage V of these two species, is not present in the collection.

Variations in the size of the leptocephalus have been noticed both in the larval and metamorphosing stages and the largest specimen in the collection measures 102·5 mm. while the smallest is only 70·5 mm. Both these larvæ are edentulous indicating the commencement of metamorphosis. It is likely that ecological and environmental factors, rather than size, influence the metamorphosis of these larvæ. High variation in myotome number is found in this species and it ranges from 142 to 152 with the majority of the specimens showing 146 myotomes.

Stage I (Text-Fig. 1 and Photograph 1).—There are 6 specimens in the collection which come under this stage and all of them possess the full set of larval teeth. The different specimens show slight variations in the myotome number and in the position of the anal opening. This stage represents the full-grown leptocephalus.

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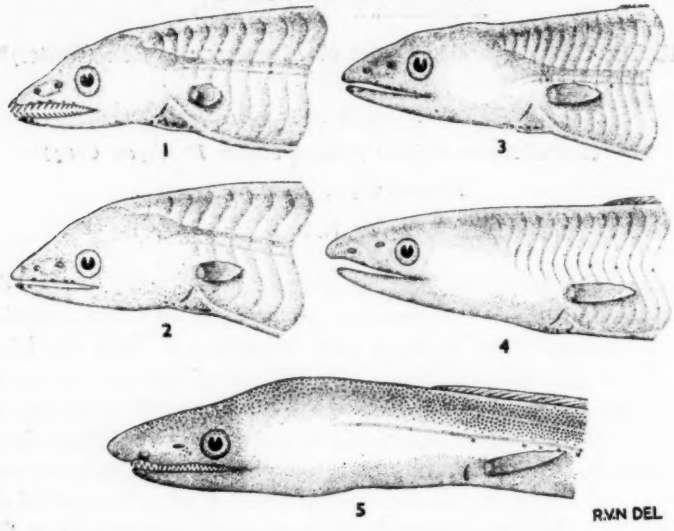
The leptocephalus is of moderate length with a tapering body. The maximum height is in the region of the vent and it measures 7·9 mm. which is 10·9 times in length.



TEXT-FIGS. 1–5. Head region of the metamorphosing stages of the leptocephalus of *Muraenesox talabon*, ×*ca.* 5.

The head is short and measures 4·8 mm. which is 17·9 times in length. The snout is long and sharply pointed and it is 2·4 times in head. The eye is oval in shape and its diameter is 5·3 times in head. The nostrils have become clearly differentiated and the anterior nostril is situated in the anterior third of the snout while the posterior one is in the middle of the interspace between the anterior nostril and the eye. The cleft of the mouth is straight and extends up to and sometimes slightly behind the posterior border of the eye. Both the jaws are of the same length and are provided with moderately long and pointed teeth which are directed forwards. The teeth formula is $1 + 11 + 6/1 + 16$. Each half of the upper jaw is provided with 18 teeth which are of unequal size. The first one is the grasping tooth which is thin, relatively short and originates slightly above the tip of the snout. This tooth is followed by a group of 11 long teeth of which the first one is the longest and it is larger than the grasping tooth. The other teeth in this group become shorter backwards and the last one is about $\frac{1}{4}$ the length of the first tooth. The second group is composed of the six remaining teeth which are short, conical and of

uniform size. These teeth are compactly arranged near the posterior border of the upper jaw. The lower jaw is provided with 17 teeth in each half and they are regularly arranged and equidistant from one another. The grasping tooth originates slightly below the tip of the lower jaw. It is long, curved and of about the same size as the adjacent teeth in the lower jaw. The other teeth become shorter in size backwards with the last one appearing as a short conical projection only.

TABLE I

Measurements of Murænesox talabon

	Stage I	Stage II	Stage III	Stage IV	Stage V
Total length ..	86·0	87·0	85·5	78·0	84·5
Maximum height ..	7·9 (9·2)	8·1 (9·3)	6·7 (7·8)	5·9 (7·6)	2·8* (3·3)
Predorsal distance ..	21·9 (25·5)	13·0 (14·9)	9·1 (10·6)	9·0 (11·5)	10·5 (12·4)
Preanal distance ..	59·0 (68·6)	45·7 (52·5)	32·4 (37·9)	28·6 (36·7)	28·8 (34·1)
Length of head ..	4·8 (5·6)	5·9 (6·8)	6·2 (7·3)	8·0 (10·3)	11·5 (13·6)
Length of snout ..	2·0 (41·7)	2·2 (37·3)	2·3 (37·1)	2·3 (28·8)	2·9 (25·2)
Diameter of eye ..	0·9 (18·8)	0·8 (13·6)	0·8 (12·9)	0·8 (10·0)	0·9 (7·8)
Total myotomes ..	142	150	147	148	..
Preanal myotomes ..	80	69	52	48	..
Predorsal myotomes ..	30	17	12	10	..

* Height of head is 3·8 mm.

The alimentary canal is moderately long, straight and extends nearly $\frac{2}{3}$ the length of the larva. The type larva shows 142 myotomes with the vent below the 80th myotome. The preanal distance is long and it is 1·5 times in length. The dorsal fin is very long and is about $\frac{3}{4}$ the length of the larva with the predorsal distance 3·9 times in length.

The pectoral fin is oval and shows only indistinct rays. The dorsal and the anal fins are well formed with 252 and 216 rays respectively. The

posteriormost rays of both these fins are longer than the rest and are of about the same length as those of the caudal fin. The caudal fin which is continuous with the other vertical fins shows 12 distinct rays.

The larval pigmentation exhibits characteristic features which are of considerable importance in distinguishing this leptocephalus from those of the other two species of *Muræsox*. The head pigmentation consists of 4–5 unbranched black chromatophores arranged along the edge of the upper jaw and a row of 6–7 similar large pigment cells in the heart region. In most of the specimens 1–2 highly branching black chromatophores are present at the tip of the lower jaw. The mid-lateral row of chromatophores, situated below the vertebral column, commences usually from the 14th myotome and extends up to the Caudal region. The pigment cells are circular in shape in the anterior region becoming linear in the posterior region of the body. Two to three chromatophores are occasionally present in front of and in a line with this row at intervals of 2–3 myotomes. Slight variations in this general arrangement have been noticed and in a few specimens some of the posterior myotomes are not provided with pigment cells and show occasional breaks in the regularity of their arrangement. The pigmentation of the alimentary canal is very striking and constitutes an important diagnostic feature of the larva and this is observed to persist in the metamorphosing and elver stages. The salient feature of the gut pigmentation is the asymmetrical distribution of the chromatophores. On the ventral side of the gut is a pair of irregular rows of elongated, unbranched black chromatophores extending throughout the posterior $\frac{2}{3}$ of the length of the alimentary canal. In addition to this paired row, there is another prominent row of pigment cells found on the right side of the gut and this row is situated above the right paired row of chromatophores (Photograph 2). This lateral row is composed of compactly arranged, small unbranched chromatophores which become prominent near the anal opening. In some specimens this unpaired row appears as a black line. In most of the specimens this row is seen distinctly as a dotted line extending anteriorly up to the liver diverticulum while the paired rows of pigment cells tend to break up in the anterior region.

The pectoral and dorsal fins are unpigmented while the caudal and anal fins are provided with a few scattered black chromatophores at their bases. The anal fin is also provided with numerous, elongated black pigment cells which are uniformly arranged and usually 2–3 chromatophores are found in a line along the anterior border of each of the anal fin rays. A few black chromatophores are also found scattered amidst the caudal fin rays, especially in the lower half of the fin.

Stage II (Text-Fig. 2 and Photograph 1).—This is the edentulous stage showing the commencement of metamorphosis and apart from the loss of the larval set of teeth a few other changes are also seen in this stage.

There is no change in the general appearance of the larva and the specimen described here is slightly longer than the previous stage and measures 87 mm. in length and 8·1 mm. in height. Consequently the latter is only 10·7 times in the former showing a slightly lower proportion than that of the leptocephalus. The head has elongated and measures 5·9 mm. and it is 14·7 times in length. The snout has also become longer and it is 2·7 times in head. The eye is slightly smaller and its diameter is 7·4 times in head.

The type larva described here shows 150 myotomes of which 69 are preanal in position and the preanal distance is 1·9 times in length. A similar shifting of the origin of the dorsal fin is seen and it is above the 17th myotome in this stage. The predorsal distance is reduced considerably and it is 6·7 times in length.

The pectoral fin is elongated and pointed in this stage. There is no change in the pigmentation of the larva.

Stage III (Text-Fig. 3 and Photograph 1). — The larva has become opaque due to the reduction in the height and the marked increase in the thickness and it measures 85·5 mm. in length. The maximum height of 6·7 mm. is in the middle of the body and it is 12·8 times in length.

The head is long and measures 6·2 mm. which is 13·8 times in length The snout is 2·7 times in head. There is no change in the size of the eye and its proportion has increased to 7·8 times in head.

The myotomes are fairly distinct and the type larva possesses 147 muscle segments of which 52 are preanal in position. The alimentary canal has become shorter and it extends a little more than the anterior third of the larva with the preanal distance 2·6 times in length. The origin of the dorsal fin has also moved forwards and it is situated above the 12th myotome resulting in a further reduction of the predorsal distance which is 9·4 times in length.

The pectoral fin has become longer and shows distinct rays. No change is observed in the vertical fins.

There is no difference in the general pigmentation, but due to the opaqueness of the larva, the pigment cells in the heart region are somewhat indistinct in this stage. Due to the reduction in the length of the alimentary canal, the paired and unpaired rows of chromatophores present on it have become shorter with the pigment cells showing a more compact arrangement. The chromatophores of the unpaired row have accumulated in front of the anus

in an irregular manner giving a stippled appearance to this region. The pigment cells of the paired rows also show a similar tendency to crowd near the anus. The concentration of pigment cells on either side of the anus and in front of it is distinctly seen when the larva is viewed from the ventral side.

Stage IV (Text-Fig. 4 and Photograph 1).—The larva in this stage is perfectly opaque due to further reduction in height and increase in thickness. The leaf-like shape of the larva is almost lost and the cylindrical shape of the elver is attained in this stage. The type specimen measures 78 mm. in length. The maximum height of 5·9 mm. is seen just behind the vent and it is 13·2 times in length.

The head shows considerable elongation in length, particularly in the post-orbital region. The larval shape of the head is lost in this stage and the head resembles that of the adult. The head is 8 mm. long and it is 9·8 times in length. Even though the snout does not show any increase in length, its proportion in head has increased to 3·5 times. The size of the eye remains unchanged and it is 10 times in head. The tip of the snout projects slightly beyond that of the lower jaw and the adult set of teeth has begun to appear in the form of very minute projections on both the jaws.

The myotomes are not very distinct, however, they could be counted under reflected light and about 148 muscle segments are present in the type specimen. The anus occupies a forward position and it is under the 48th myotome. A proportionate decrease in the postanal distance is seen and it is 2·7 times in length. The origin of the dorsal fin also shows a slight forward movement and it is above the 10th myotome. The predorsal distance is 8·7 times in length.

The pectoral fin has become long and pointed while the median fins do not show any change in their shape.

There is no change in the colouration of the larva. The pigmentation has become somewhat faint, especially the mid-lateral row of chromatophores. The accumulation of pigment cells on either side of the anus is seen conspicuously in this stage.

Stage V (Text-Fig. 5 and Photograph 1).—This is the elver stage comparable to Stage VI in the metamorphosis of *Muræsox cinereus* and *Muræsox talabonoides* and no metamorphosing larva corresponding to Stage V in the metamorphosis of these species is available in the collection. The elvers are represented by two specimens which are of about the same size and appearance. The metamorphosis is almost complete and all the characters of the adult, excepting the colouration, are seen distinctly in the elver.

The body has become perfectly cylindrical and the maximum height is seen in the anterior region and measures 2·8 mm. The height is 30·2 times in length and this proportion is about 3 times that of the larva. It may be mentioned here that the height of the head is greater than that of the body and measures 3·8 mm. In this respect the elver of this species differs from those of the other two species in possessing a large head and a slender body.

The head, which has undergone considerable changes in shape, is very long and resembles the adult head in every detail. The head measures 11·5 mm. and it is only 7·3 times in length. The snout has also become longer and its tip projects considerably beyond that of the lower jaw and the characteristic rostral constriction is seen distinctly. The snout is 4 times in head. The eye has become slightly larger, but its diameter is contained 12·8 times in head and this is due to the lengthening of the head. The anterior nostril is tubulate and is situated at the anterior third of the snout very near the border of the upper jaw while the posterior one appears as slit-like aperture situated in front of the eye by about half its diameter. The gape of the mouth extends beyond the eye. The teeth are well developed in both the jaws and they are pointed, incurved and closely arranged.

The dorsal fin takes its origin far in front of the gill opening. The predorsal distance is greater than in the preceding two stages and it is 8 times in length. The anus is situated at the anterior third of the body and the preanal distance is 2·9 times in length.

The fins are well developed and the pectoral fin is very long and pointed resembling that of the elver of *M. talabonoides*. The dorsal and anal fins have become slightly wider and shows the rays very clearly.

Indications of the adult colouration are seen in the elver and numerous, small, diffusely branching brown pigment cells have appeared on the dorsal side of the head especially in the inter-orbital and post-orbital regions. Similar chromatophores are also present on the dorsal side of the snout, but show a diffuse and scattered arrangement. A few such pigment cells are also present in front of the eye. The pigmentation of the body is confined to the dorsal side only and it extends on the sides up to the lateral line. On the body the chromatophores are arranged uniformly leaving two linear unpigmented zones; one situated at the base of the dorsal fin and the other midway between the dorsal fin and the lateral line. These unpigmented streaks extend throughout the length of the elver and appear as white lines against the brown background. The larval pigmentation persists in the elver and the chromatophores of the mid-lateral series and those of the anal fin are conspicuously seen. The pigmentation of the alimentary canal is also

prominent and appears in the form of an irregular line along the posterior half of the gut and also in the form of a dark line surrounding the anal opening.

The other elver present in the collection shows a deeper pigmentation, even though it is of the same size as the type described here. The pigmentation on the dorsal side of this elver is intense and along the edges of the dorsal and anal fins numerous highly branching, compactly arranged, brown pigment cells have appeared giving a dark-brown colour to their borders.

REMARKS

The leptocephalus of *Muræsox talabon* exhibits characters which are different from those of the other two species of the genus, namely, *Muræsox cinereus* (Nair, 1947) and *Muræsox talabonoides* (Nair and Mohamed, 1960 *a*). Table II, which gives the characters of the three larvæ, shows that

TABLE II

Measurements of the leptocephali of Muræsox

	Leptocephalus of *Muræsox cinereus* Nair, 1947	Leptocephalus of *Muræsox talabonoides* Nair and Mohamed, 1960 *a*	Leptocephalus of *Muræsox talabon*
Total length	81·0	81·0	86·0
Maximum height	11·0 (13·6)	6·2 (7·7)	7·9 (9·2)
Predorsal distance	20·0 (24·7)	13·8 (17·0)	21·9 (25·5)
Preanal distance	53·0 (65·4)	46·2 (57·0)	59·0 (68·6)
Length of head	5·0 (6·2)	5·3 (6·5)	4·8 (5·6)
Length of snout	2·2* (44·0)	1·9 (35·8)	2·0 (41·7)
Diameter of eye	0·7* (14·0)	0·9 (17·0)	0·9 (18·8)
Total myotomes	138	149	142
Preanal myotomes	78	74	80
Teeth	15/15*	20/17	18/17

* Calculated from the Text-Figures.

the present larva is of medium height only while those of *M. cinereus* and *M. talabonoides* have the maximum and minimum heights respectively. *M. talabon* shows a similar intermediate condition in the length of the snout also which is relatively long in *M. cinereus* and short in *M. talabonoides*. The head is of moderate length in *M. cinereus* while it is long in *M. talabonoides* and short in *M. talabon*. *M. talabon* possesses the largest eye while that of *M. talabonoides* is slightly smaller with that of *M. cinereus* the smallest among the 3 species. It is interesting to point out here that the maximum number of teeth of 20/17 is seen in the shortest species, namely, *M. talabonoides* while the tallest *M. cinereus* possesses only 15/15 teeth with the medium-sized *M. talabon* showing an intermediate number of 18/17 teeth.

M. talabon shows a high myotome variation and the number varies from 142 to 152. *M. talabonoides* shows only a variation of 2 myotomes from 147 to 149 while this is not recorded in the case of *M. cinereus*, which shows the lowest number of 138 myotomes. Corresponding with the differences in the length of the alimentary canal, the position of the vent also shows variations in relation to myotome number and it is located below the 80th, 78th and 74th myotomes in *M. talabon*, *M. cinereus* and *M. talabonoides* respectively. The predorsal and preanal distances of *M. talabon* are longer than those of *M. cinereus* while these are very short in *M. talabonoides*.

The shape of the pectoral fin has some diagnostic significance, and it is circular in *M. cinereus* while it is oval in *M. talabon* and long and pointed in *M. talabonoides*.

Apart from these differences in the general and morphometric characters, the noteworthy difference is seen in the pigmentation of the 3 larvæ. The head pigmentation of these larvæ is more or less uniform; the chromatophores being present in the middle portion of the upper jaw and in the region of the heart. In addition to this normal pigmentation of the head, both *M. talabon* and *M. talabonoides* show pigment cells at the tip of the lower jaw while it is unpigmented in *M. cinereus*. There is close similarity in the pigmentation along the mid-lateral line in *M. cinereus* and *M. talabon* and the row of pigment cells commences from the 17th and 14th myotomes respectively with all the successive myotomes in both the species being provided with chromatophores. On the other hand, in *M. talabonoides* this row commences from the 23rd myotome and the pigment cells are generally distributed at intervals of 3–4 myotomes in the anterior region while posteriorly alternate myotomes alone are provided with chromatophores.

The most striking difference is seen in the pigmentation of the alimentary canal of the 3 species. In *M. talabon*, the chromatophores are arranged in

3 rows in the posterior region of the alimentary canal of which 2 are paired while the third is unpaired and found on the right side of the alimentary canal. This asymmetrical arrangement of the chromatophores in *M. talabon* is found to be constant and serves as an important character in the identification of the larval, metamorphosing and elver stages. *M. talabonoides* also exhibits a highly characteristic pigmentation of the alimentary canal and the pigment cells are grouped at definite intervals to form conspicuous dark patches which persist in the metamorphosing and elver stages. In *M. cinereus*, the pigment cells do not show any pattern in their arrangement and they are distributed along the entire length of the alimentary canal. This study of the colouration of the 3 larval species of the same genus has shown clearly that each species shows a characteristic pattern of pigmentation of taxonomic importance using which the different larvæ, metamorphosing stages and even advanced elvers could be identified easily.

Jones and Pantulu (1952) gave an account of the metamorphosing stages of the talabon eel, *Muræsox talabon* collected from the Burhabulong estuary, Chandipore, on the 27th May 1951 from the stake-net catches. They described 6 stages in the metamorphosis of the larvæ and a comparison of the different stages shows that they do not conform to the stages proposed by Schmidt (1906) and Jespersen (1942). The different stages described by them do not correspond with the metamorphosing stages of *M. cinereus* (Nair, 1947). Consequently a rearrangement of the stages is found necessary for a comparison with those of *M. cinereus* and *M. talabonoides* (Nair and Mohamed, 1960 *a*). Stage I described by Jones and Pantulu is edentulous and is comparable to Stage II of *M. cinereus* while the larva collected from Visakhapatnam and described in the addendum approaches Stage I of *M. cinereus*, even though the larva has not reached full growth as shown by the undifferentiated nostril. Stages II and III generally conform with Stages III and IV of *M. cinereus* while Stages IV and V are really elvers with very little difference either in body proportions or in colouration. There is no decrease in the depth of the body and the same height of 2·07 mm. is given for both the stages. These stages correspond to Stage VI of *M. cinereus*. It is well known that the metamorphosis of leptocephalus is accompanied by considerable reduction in length and therefore the 6th stage described by them which is a juvenile eel is not considered here.

A comparison of the measurements and their percentages of the different stages as revised above given in Table III with those of the corresponding stages of *M. cinereus* (Nair and Mohamed, 1960 *a*) shows the close agreement in the morphometric characters of the larva as well as the metamorphosing stages.

TABLE III

Measurements of Muræsox talabon (*Jones and Pantulu*, 1952)

	Larva (I)	Stage I (II)	Stage II (III)	Stage III (IV)	Stage IV (VI)	Stage V (VI)	Stage VI (Juvenile)
Total length ..	64·0	65·0	69·0	61·0	72·0	71·0	216·0
Maximum height (excluding fins)	10·5 (16·4)	5·9 (9·1)	5·9 (8·6)	3·6 (5·9)	2·1 (2·9)	2·1 (3·0)	8·5 (3·9)
Predorsal distance ..	15·5 (24·2)	9·0 (13·8)	9·5 (13·8)	7·4 (12·1)	9·9 (13·8)	9·5 (13·4)	25·5 (11·8)
Preanal distance ..	41·0 (64·1)	32·5 (50·0)	25·0 (36·2)	22·0 (36·1)	22·0 (30·6)	21·0 (29·6)	74·0 (34·3)
Length of head ..	5·0 (7·8)	5·6 (8·6)	7·8 (11·3)	7·0 (11·5)	10·5 (14·6)	10·7 (15·1)	31·0 (14·4)
Length of snout ..	2·0 (40·0)	2·1 (37·5)	2·2 (28·2)	2·3 (32·9)	2·7 (25·7)	2·7 (25·2)	7·0 (22·6)
Diameter of eye ..	0·6 (12·0)	0·6 (10·7)	0·7 (9·0)	0·6 (8·6)	0·9 (8·6)	0·9 (8·4)	2·5 (8·1)
Total myotomes ..	136	136	136	..	..	136*	136*
Preanal myotomes ..	75	58	43	..	..	34*	32*

* Vertebrae.

and this resemblance makes it necessary to examine the characters on which Jones and Pantulu have distinguished the talabon larva from that of *M. cinereus*.

The authors found that the 'metamorphosing stages of the talabon present a close similarity to those of *M. cinereus*' and pointed out a few differences between the two species. One of the characters mentioned by them is in regard to the cleft of the mouth and they state that "the cleft of the mouth in *M. cinereus* extends up to the posterior border of the eye whereas in talabon it reaches well beyond the orbit". But in the description of the Visakhapatnam larva they have stated that the "cleft of the mouth reaches the posterior border of the obrit". Even though this character has no taxonomic significance it is seen that the cleft of the mouth of the larva of talabon eel does not show any difference from that of *M. cinereus*. The second character is in regard to the myotome number which is 138 in *M. cinereus* and 136 in the talabon larva and as shown in a subsequent part of this series (Nair and Mohamed, 1960 *b*) this difference in the myotome number is not significant enough to exclude the normal variation found within a species. The third point of difference relates to the mid-lateral row of pigment cells which in the larva described by them commences from the 10th myotome while in *M. cinereus* it begins regularly from the 17th myotome only. Even though this difference is only by a few myotomes, it may be pointed out that in *M. cinereus* 3–4 chromatophores have been noticed in the preceding myotomes also and in a line with the pigment cells of the mid-lateral series. Slight individual variations have also been observed in regard to the mid-lateral pigmentation making it of doubtful utility in identification, especially when the difference is only by a few myotomes. The other differences mentioned by Jones and Pantulu are the prominent rostral constriction and the tubulate nature of the anterior nostril of the talabon larva. These characters are not dealt with in the description of the different metamorphosing stages of *M. cinereus*; however, the figures of the head region of the different metamorphosing stages show these characters clearly. The rostral constriction in *M. cinereus* is seen distinctly in the advanced metamorphosing stages, especially in Stage V while the tubular nature of the anterior nostril is also shown clearly in the figure of the head of the elver.

It is seen from the above discussion that the larva and metamorphosing stages of *M. talabon* described by Jones and Pantulu belong to *M. cinereus* in view of the almost perfect agreement in the different characters. Additional evidence in support of this assumption is given by the fact that the pectoral fin is circular in the talabon larva also and it becomes oval in the metamorphosing and elver stages and this condition is found only in

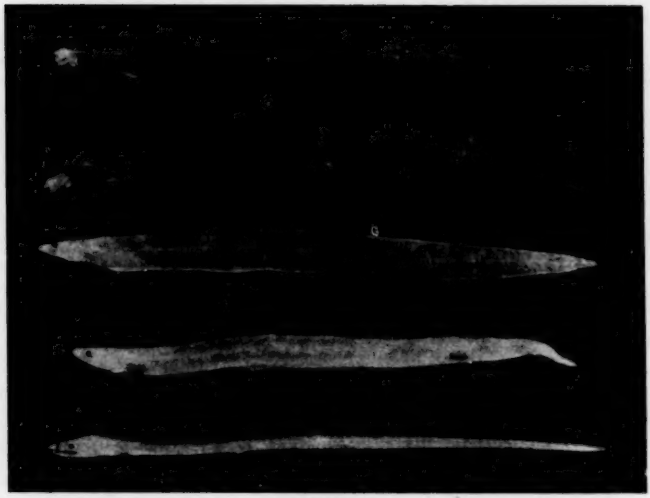


PHOTO. 1

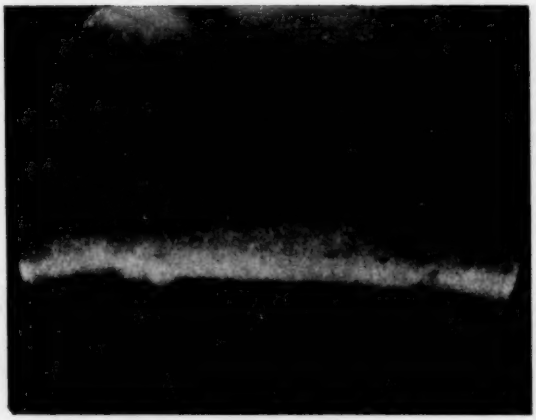


PHOTO. 2

M. cinereus. Further, the larva described by them resembles that of *M. cinereus* in the early acquisition of the adult colouration of the body during the metamorphosis itself while in the other two species this is seen only in the elver stage. Lastly it may be pointed out here that according to Jones and Pantulu, the adult of *M. cinereus* is fairly common along the Orissa and Bengal coasts.

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Explanation of Plate

PHOTOGRAPH 1. The five stages in the metamorphosis of *Muræsox talabon*. About natural size.

PHOTOGRAPH 2. A portion of the asymmetrical row of pigment cells on the alimentary canal of *Muræsox talabon*.

STUDIES ON THE LEPTOCEPHALI OF BOMBAY WATERS

III. The Metamorphosing Stages of *Uroconger lepturus* (Richardson)*

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THE leptocephalus of *Uroconger lepturus* was first described by Nair in 1946 and its occurrence was later recorded from the Gulf of Mannar (Nair, 1948). The present collection consists of several leptocephali and elvers of different sizes, the majority of which was collected from Versova (*vide* Introduction, Nair and Mohamed, 1960). All the stages described here were taken from the collection made on the 9th January 1954. Most of the larvæ in the collection were edentulous and showed high variation in the preanal myotome number and the latter character was also found to vary in the few toothed larvæ present in the collection.

Stage A (Text-Fig. 1).—The collection contains a few specimens belonging to this stage and all of them show the full set of larval teeth including the grasping tooth. This stage, which represents a growing larva, is younger than the leptocephalus described by Nair (1946) and differs from the latter in the possession of the grasping tooth and in the backward position of the vent which indicate that the Madras larva has reached full growth prior to metamorphosis. The growing larva also shows variations in the number of preanal myotomes indicating that the migration of the anus takes place in the growing phase itself. The youngest of the toothed larvæ showing the maximum number of preanal myotomes is described here as the type of this stage.

The larva is transparent and long and measures 110 mm. in length. It is moderately high and the maximum height of 6·2 mm. is seen in the middle region and it is 17·7 times in length. The leptocephalus of this species collected from Madras and Gulf of Mannar were considerably higher and the

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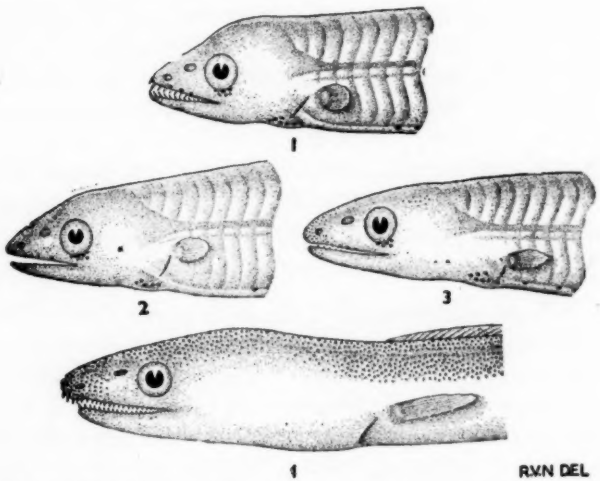
height was 10·2 times in length. This difference in the height of the larva collected from the various places is believed to be caused by the environmental factors. The body tapers gradually towards the head and the tail and the latter is long, pointed and whip-like in appearance. The tail shows a tendency to break off owing to its slender and thread-like nature and the collection contains quite a few larvæ with incomplete tail and some showing its regeneration. Elvers showing regenerated tail have been frequently met with and it was found difficult to distinguish the normal ones from those possessing regenerated tails without staining in alizarin. This partly accounts for the high variation in the vertebral and fin ray counts observed in the elvers and the juveniles of this species.



TEXT-FIGS. 1–4. Head region of the growing and metamorphosing stages of the leptocephalus of *Uroconger lepturus*, ×*ca.* 4·5.

The head is very short and measures 5 mm. which is 22 times in length. The snout is moderately pointed and it is 2·9 times in head. The nostrils are of unequal size and the anterior one is small and situated near the tip of the snout while the posterior one is large and nearer to the front margin of the eye than to the anterior nostril. The eye is large, prominent and slightly oval in shape and its diameter is 3·8 times in head. The jaws are of the same length and the cleft of the mouth extends to a level with the centre of the eye. The jaws are provided with pointed forwardly directed teeth and the dental formula is 1 + 1 + 6 + 15/1 + 14. Each half of the upper jaw carries 23 teeth. The first is the short grasping tooth placed at the tip of the

snout and this is followed by a long and strong tooth. The other teeth are arranged in two groups. The first group is composed of 6 moderately long pointed teeth while the remaining 15 teeth forming the second group are very small and closely arranged in the posterior half of the jaw. The lower jaw possesses in each half a curved grasping tooth placed slightly below its tip and 14 teeth which are placed equidistant from one another. These teeth are fairly long and pointed and become shorter backwards.

The myotomes are distinct except in the whip-like portion of the tail where they are somewhat faint and could be made out satisfactorily after staining with eosin. Altogether 218 myotomes are present in the type specimen and variation from 204–223 has been observed in this species. The alimentary canal is very long, straight and extends nearly $\frac{3}{4}$ the length of the larva with the anus situated below the 122nd myotome. The origin of the dorsal is nearer to the snout than to the caudal and the predorsal distance is 2·4 times in length. The preanal distance is only 1·3 times in length.

The anal fin is very short and both the anal and dorsal fins are continuous with the caudal and the rays of the caudal fin are about double the size of those of the other fins. The pectoral fin is circular in shape with indistinct fin rays.

Even though the pigmentation of the larva is very feeble, the chromatophores are very large and conspicuous and they are observed to persist in the metamorphosing and elver stages. Along the edge of the upper jaw 3–4 circular, unbranched, chromatophores are arranged in a line. A group of black branching chromatophores are present just below the eye and this pigmentation, as pointed out by Nair (1946), is a distinguishing feature of the larva of this eel. Near the heart 4–7 large black pigment cells are found scattered without any definite arrangement.

The mid-lateral row of chromatophores usually commences from the 12th myotome and the pigment cells which are large, black and unbranched are regularly distributed in each of the succeeding myotomes and occasionally at intervals of 1–4 muscle segments. This row does not extend up to the whip-like portion of the tail. A row of closely arranged black pigment cells is found along the dorsal side of the alimentary canal and this series extends beyond the vent with the chromatophores arranged along the base of the anal fin. The post-anal chromatophores of this row are linear and show a scattered distribution and are not found in the tail region. The other fins do not show any pigmentation.

Stage I (Text-Fig. 2 and Photograph 1).—Since the stage described in 1946 represents the full-grown leptocephalus comparable to Stage I in the metamorphosis of *Muraenesox cinereus* (Nair, 1947) and as no significant difference has been observed in similar stages present in the Bombay collection, the leptocephalus stage is not described here. For comparison, the general and morphometric characters of the Madras larva are also included in Table I along with those of the different stages described here.

TABLE I

Measurements of Uroconger lepturus

	Stage A	Stage I	Stage II	Stage III	Larva Nair, 1946
Total length ..	110·0	103·0	98·0	88·0	112·0
Maximum height ..	6·2 (5·6)	6·7 (6·5)	4·6 (4·7)	3·8 (4·3)	11·0 (9·8)
Predorsal distance ..	45·0 (40·9)	27·6 (26·8)	11·0 (11·2)	11·5 (13·1)	43·0 (38·4)
Preanal distance ..	82·2 (74·7)	55·0 (53·4)	32·0 (32·7)	27·5 (31·3)	82·0 (73·2)
Length of head ..	5·0 (4·5)	5·4 (5·2)	6·9 (7·0)	10·8 (12·3)	6·0 (5·4)
Length of snout ..	1·7 (34·0)	1·9 (35·2)	2·1 (30·4)	2·8 (25·9)	2·1* (35·0)
Diameter of eye ..	1·3 (26·0)	1·3 (24·1)	1·3 (18·8)	1·3 (12·0)	1·6* (26·7)
Total myotomes ..	218	206	204	212†	216
Preanal myotomes ..	122	79	52	44†	116
Predorsal myotomes ..	57	40	14	10	..

* Calculated from the Text-Figure.
† Vertebra(e).

This stage represents the edentulous condition in the metamorphosis of the larva and it is comparable to Stage II of *M. cinereus*. The larva measures 103 mm. in length. The maximum height is seen in the middle region and it measures 6·7 mm. which is 15·4 times in length. The height gradually decreases at the two extremities and this is more pronounced in the posterior region which ends in a finely pointed thread-like tail.

The head has grown longer and measures 5·4 mm. and it is 19·1 times in length. A slight increase in the length of the snout is seen and it is 2·8 times in head. There is no change in the size or shape of the eye and it is 4·2 times in head. In almost all the specimens belonging to this stage, the tail was found to be regenerated and as such it was found impossible to get the correct number of muscle segments and the fin rays. The type specimen shows only 206 myotomes. The important difference seen in the edentulous stage is the shifting of the origin of the dorsal fin and the anus. The origin of the dorsal fin is situated above the 40th myotome in this stage and consequently the predorsal space is reduced considerably and it is 3·7 times in length. Marked reduction in the length of the alimentary canal is also noticed and the anus is situated just behind the middle of the larva below the 79th myotome. The preanal distance is 1·9 times in length.

The fin rays are distinctly seen in the vertical fins and their number as stated earlier is found to be highly variable.

There is no change in the pigmentation of the larva in this stage.

Stage II (Text-Fig. 3 and Photograph 1).—This stage which corresponds to Stage III of *Muraenesox cinereus* is represented in the collection by a single specimen. The larva has becom eopaque and shows reduction in height and moderate increase in thickness. The larva measures 98 mm. and the height which is 4·6 mm. is 21·3 times in length. The reduction in the height is very conspicuous in the posterior half of the larva while the anterior half is more or less uniform in height.

The head has elongated considerably, especially in the post-orbital region and measures 6·9 mm. which is 14·2 times in length. The snout has also become longer and it is 3·3 times in head. The eye in this stage does not show any change in size and it is 5·3 times in head. The adult set of teeth has begun to develop and they are seen as small pointed projections which are seen better in the lower jaw.

The myotomes have become almost indistinct in the posterior region due to the reduction in the height and the thickening of the larva, while those of the anterior region could be made out faintly. The type specimen possesses 204 myotomes. The anus has shifted further anteriorly and it is opposite the 52nd myotome with the preanal distance 3·1 times in length. The origin of the dorsal fin has also shifted considerably forwards and it is above the 14th myotome with the predorsal distance 8·9 times in length.

Changes in the pigmentation of the larva indicating the gradual assumption of the adult colouration is seen in this stage. The tips of the snout

and the lower jaw have become powdered with minute black chromatophores. On the dorsal side of the post-orbital region a group of small black chromatophores is present. The body pigmentation does not show any change. The chromatophores of the mid-lateral series have become very prominent with ramifying branches. The pigment cells along the alimentary canal and the base of the anal fin have also become larger and dendritic and this row extends up to the tip of the tail in this stage.

Stage III (Text-Fig. 4 and Photograph 1).—This is the final elver stage in the metamorphosis of the larva comparable to Stage VI of *Muræsox cinereus*. The elver is perfectly cylindrical and measures only 88 mm. in length. The height is considerably reduced and it is 3·8 mm. which is 23·2 times in length. The maximum height is seen just behind the gill opening and the body gradually tapers from this point to terminate in the finely pointed tail.

The length of the head has increased to 10·8 mm. and it is only 8·1 times in length. The snout, which resembles that of the adult, is long and projects beyond the tip of the lower jaw and it is 3·9 times in head. The eye has become perfectly circular and it is 8·3 times in head. Most of the adult teeth have appeared in the elver and both the jaws show rows of small, sharply pointed and incurved teeth.

Alizarin preparation of the elver shows the presence of 212 vertebræ. Further reduction in the length of the alimentary canal has taken place and the anus is situated below the 44th vertebra with the preanal distance 3·2 times in length. The origin of the dorsal fin is above the 10th vertebra and slightly behind that of the pectoral fin. In spite of the anterior shifting of the origin of the dorsal fin, the predorsal distance is greater than that of the previous stage and this is due to the marked elongation of the head of the elver which is 7·7 times in length.

Important changes in the pigmentation have taken place in the elver which still retains the larval pigmentation, especially the mid-lateral row of chromatophores. The ventral pigmentation, on the other hand, has become somewhat faint due to the opacity of the elver. Numerous, small, dendritic uniformly sized and closely arranged chromatophores have appeared on the dorsal side of the head and the body giving a uniform brown colour to the dorsal side of the elver. On the head, the pigmentation is confined to the inter-orbital region while on the body, it extends laterally up to the middle of the space between the base of the dorsal fin and the lateral line. In the posterior region, the body pigmentation extends below the lateral line also and it is intense near the caudal region. An unpigmented narrow zone,

which appears as a white line to the naked eye, is present on either side of the anterior $\frac{2}{3}$ of the dorsal fin.

Along the border of the dorsal and anal fins, numerous dark brown pigment cells have appeared giving it a dark brown colour which is deeper in the tail region.

Several elvers in different stages of growth are present in the collection and they do not show any appreciable change in the morphometric characters. Intensification of the pigmentation is noticed during the growth of the elver and its body assumes a uniform brown colour while the edges of the vertical fins are black in colour.

REMARKS

It was pointed out that Stage A described here is an advanced growing stage, while the larva described by Nair in 1946 represents the full-grown leptocephalus of *Uroconger lepturus*. Three stages in the metamorphosis of the leptocephalus alone are available in the Bombay collection. For studying the changes undergone during the growing phase, a comparison of the growing Stage A with the full-grown leptocephalus described in 1946 is not attempted here, since there is only one stage belonging to the former category. Further, these larvæ are collected from widely separated places like Madras and Bombay which present different ecological conditions and, therefore, a comparison of the morphometric characters is not considered desirable. However, it is significant to note that in *U. lepturus* also the migration of the anus and the shifting of the origin of the dorsal fin take place in the larva even before it reaches the full growth, thus resembling the condition found in *Muræsox talabonoides* (Nair and Mohamed, 1960).

Most of the larvæ in the collection are without teeth and while describing the Madras larva Nair pointed out that " in the majority of the examples collected the larval set of teeth has dropped off and indications of the adult set are seen as minute conical projections". A similar condition was noted in the leptocephalus collected from the Gulf of Mannar and Nair (1948) stated that " all the larvæ in the present collection are edentulous with great reduction in the length of the alimentary canal". The abundance of the edentulous stage in these centres like Madras, Gulf of Mannar and Bombay suggests that the leptocephalus of *U. lepturus* generally enters the foreshore waters after the commencement of metamorphosis which is indicated by the loss of the larval set of teeth and preceded by anal migration.

It may be mentioned here that the transforming stages of this eel have been found to be extremely rare in the ' dol ' net catches, even though the

larvæ and the elvers have been collected in large numbers. Similarly the collections made at Madras and the Gulf of Mannar also do not contain any metamorphosing or elver stages. It is likely that the metamorphosing stages are found in the relatively deeper waters and they also manage to dodge the net effectively because of their greater agility. The occurrence of the elvers in large numbers in the Bombay collection is perhaps due to the fact that they are voracious feeders and go after prey like *Acetes* and shrimps which occur in enormous numbers in the Bombay waters during this period and are trapped in the 'dol' nets along with them.

The metamorphosis of *U. lepturus* shows the usual changes observed during the metamorphosis of the other Indian leptocephali. The reduction in the height observed in this species is by 56·1% of the larval height while the predorsal and preanal distances are reduced by 65·9% and 57·2% respectively. The length of the head of the elver is more than 2¼ times the length of the head of the leptocephalus. Due to this increase in the head length, the percentage values of the snout and the eye are reduced considerably. The formation of the adult colouration is seen even in Stage II while in the elver, the dorsal side is uniformly brown and the fins have a dark brown border.

The resemblance in the general shape of the leptocephalus of *U. lepturus* to *Leptocephalus acuticaudatus* (Kaup, 1856) was pointed out by Nair (1946). Kaup's description of the larva is based on a single specimen collected by Dussumier from the Malabar coast. Bertin (1935) who re-examined the types of leptocephali described by Kaup states that 2 specimens sent by Dussumier in 1830 from the Coromandel coast are present in the Paris Museum. Apparently there is some confusion about the number and place of collection of *L. acuticaudatus*. However, according to Bertin's redescription the 2 specimens measure 137 and 135 mm. in length with a maximum height of 10 mm. The origin of the dorsal fin is nearer the snout with a preanal distance of 25 and 20 mm. respectively while the anal fin occupies half the length of the body. The larvæ possess slightly more than 200 myotomes. The tail is pointed and ends in the pointed caudal fin. Chromatophores are present along the ventral side irregularly distributed up to the base of the tail and also along the lateral line. These characters given by Bertin show that in general shape, body proportions, myotome number and also in pigmentation *L. acuticaudatus* resembles the larva of *U. lepturus* and justifies the assumption that the former is the larva of the latter which is one of the common eels of the coastal waters.

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EXPLANATION OF PLATE

PHOTOGRAPH 1. The three stages in the metamorphosis of *Uroconger lepturus*, ×*ca.* ¾.

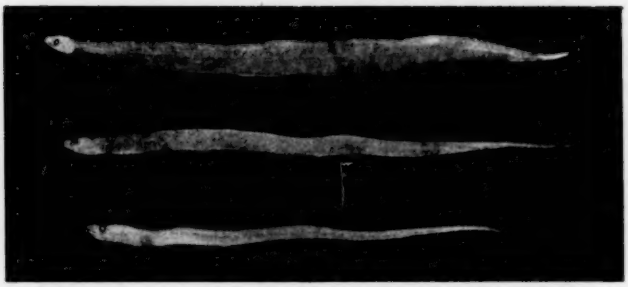

PHOTO. 1

STUDIES ON THE LEPTOCEPHALI OF BOMBAY WATERS

IV. Some Ophichthyid Leptocephali*

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Leptocephalus I

A number of specimens of this leptocephalus which belongs to the ophichthyid group were obtained from the 'dol' net catches at Versova (*vide* Introduction, Nair and Mohamed, 1960). Examination of these larvæ showed variations in the length and height in the different specimens belonging to the same stage of growth as noticed in *Muræsox talabonoides*. The collection contains several larvæ in different stages of growth and 3 typical growing stages and the full-grown leptocephalus are described here.

Stage A (Text-Fig. 1).—The youngest growing larva is represented in the collection by 4 specimens of which the smallest measures 75 mm. The larva possesses an elongated and compressed body which tapers gradually towards the head and the tail. The height is more or less uniform in the middle portion of the body and the maximum height of 4 mm. is seen in the anal region. The height is 18·8 times in length.

The head is very small and measures 3 mm. and is contained 25 times in length. The nostrils have not differentiated in this stage. The eye is very large and conspicuous with a diameter of 0·8 mm. which is 3·8 times in head. The jaws are of about the same size and the gape of the mouth extends up to the posterior third of the eye. Both the jaws are provided with strong, pointed teeth which are directed forwards and the dental formula is $1 + 6 + 8/1 + 12$. Each half of the upper jaw is provided with a strong, curved grasping tooth followed by two groups of teeth. The 6 teeth comprising the first one are uniformly long while the remaining 8 teeth belonging to the second group are conical and short. The teeth of the first group are

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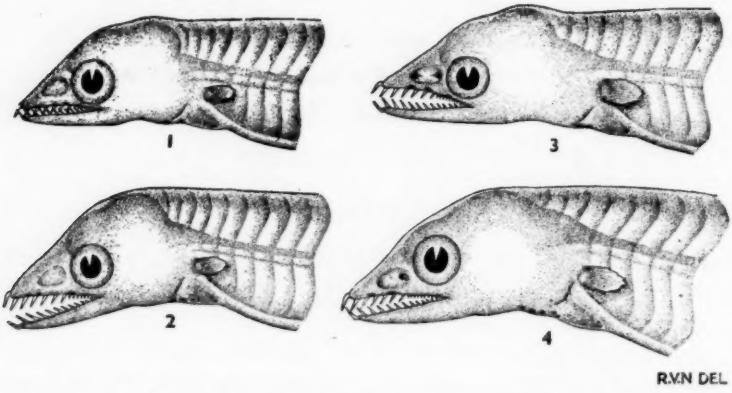
spaced wide apart while those of the second group are compactly arranged. In addition to the grasping tooth, 12 teeth are present in each half of the lower jaw and these are arranged equidistant from one another. The first tooth is the longest and the strongest while the succeeding ones become smaller backwards with the last one the shortest in the series.



TEXT-FIGS. 1–4. Head region of the growing stages of Leptocephalus I, ×*ca.* 8.

TEXT-FIGS. 5–6. Fig. 5. Head region of Leptocephalus II, ×*ca.* 6. Fig. 6. Head region of Leptocephalus III, ×*ca.* 8.

The alimentary canal is fairly long and extends up to about the middle of the larva with the anal opening below the 64th myotome. The preanal distance is 1·9 times in length. The alimentary canal shows 6 ganglion-like swellings situated more or less equidistant from one another and opposite the myotomes 11–15, 20–22, 30–31, 38–39, 47–49 and 54–55. Of these, the first two thickenings are large and prominent while the others become smaller backwards.

The pectoral fin is very small with faint indications of the rays. The dorsal fin, which is situated in the caudal region, is extremely short with the predorsal distance 1·1 times in length. The anal fin commences from about the middle of the larva and the rays are fairly distinct in both these fins. The

preanal distance is 1·9 times in length. The anal fin shows 186 rays while the dorsal fin shows variation in the fin ray count depending on the stage of growth of the larva. The tail fin is absent and the caudal portion ends in a pointed projection.

The pigmentation of the larva is very characteristic and is useful in its identification. The head pigmentation is very feeble and consists of 2–3 oblong black chromatophores near the margin of the upper jaw and 2–3 branching chromatophores in front of the heart and below the gill opening.

TABLE I

Measurements of Leptocephalus I

	Stage A	Stage B	Stage C	Stage I
Total length ..	75·0	89·5	112·0	128·0
Maximum height ..	4·0	4·2	5·2	5·3
	(5·3)	(4·7)	(4·6)	(4·1)
Predorsal distance ..	71·7	84·1	105·0	119·6
	(95·6)	(94·0)	(93·8)	(93·4)
Preanal distance ..	40·0	46·8	60·0	69·2
	(53·3)	(52·3)	(53·6)	(54·1)
Length of head ..	3·0	3·3	4·0	4·4
	(4·0)	(3·7)	(3·6)	(3·4)
Length of snout ..	1·3	1·3	1·4	1·5
	(43·3)	(39·4)	(35·0)	(34·1)
Diameter of eye ..	0·8	0·9	0·9	0·9
	(26·7)	(27·3)	(22·5)	(20·5)
Total myotomes ..	146	144	144	146
Preanal myotomes ..	64	65	65	67

A row of pigment cells is present along the mid-lateral region just below the vertebral column. The chromatophores of this series begin from the 10th myotome and all the succeeding myotomes with a few exceptions here and there are provided with chromatophores. These pigment cells are unbranched and more or less circular in the anterior region while they are oval in shape in the middle region becoming thin and linear in the posterior region. Occasionally additional chromatophores have been observed to be present

on some of the myotomes of the caudal region. In addition to this normal pigmentation, six prominent postanal groups of black chromatophores are present internally below the vertebral column and opposite myotomes 71, 86, 98, 111, 121 and 134. The mid-lateral chromatophores of these myotomes are also conspicuously larger than the others in the series and the internal pigment groups together with the prominent mid-lateral pigment cells adjoining them give a conspicuous dotted appearance to the larva.

Another distinguishing feature of the pigmentation of the larva is the accumulation of a number of branching black chromatophores above the 6 ganglion-like thickenings of the alimentary canal and on the ventral side near the anal opening. These groups appear to the naked eye as short streaks along the ventral border of the larva. This characteristic pigmentation of the larva, namely, 7 short streaks along the preanal ventral side and 6 large spots along the postanal mid-lateral line, which are arranged more or less at regular intervals, is found to be highly useful for identification.

Some irregularly arranged branching chromatophores are clustered round the tip of the tail. The dorsal fin is unpigmented while a branched chromatophore is present at the base of each of the anal fin rays excepting a few of the anteriormost rays.

Stage B (Text-Fig. 2).—This larva is slightly older than the previous stage and shows general increase in body proportions. The larva measures 89·5 mm. in length and 4·2 mm. in height which is 21·3 times in length.

The length of the head shows slight increase but not proportionate to that of the body. It measures 3·3 mm. which is 27·1 times in length. There is slight increase in the diameter of the eye and it is 3·7 times in head. The snout is 2·5 times in head and the differentiation of the nostrils is not seen in this stage also. The dentition remains unchanged except for the addition of one more tooth in each half of the upper jaw and the teeth formula is $1+6+9/1+12$.

The type specimen shows 144 myotomes and the anal opening is situated below the 65th muscle segment. There is no reduction in the predorsal and preanal distances and they continue to be 1·1 and 1·9 respectively in length.

The pectoral fin has become slightly larger in size.

A few changes in the general pigmentation of the larva have been noticed in this stage. Some more chromatophores have appeared in the head region above the œsophagus. The anterior mid-lateral chromatophores which were

circular in the previous stage have assumed an elongated and linear shape. In between the ventral preanal pigment groups other small and inconspicuous groups are occasionally present in this stage. The scattered pigment cells present at the tip of the tail have become localised in their distribution and are seen on the dorsal and ventral sides of the terminal portion of the tail.

Stage C (Text-Fig. 3).—This is the penultimate stage in the growth of the leptocephalus and it measures 112 mm. in length. Appreciable increase in the height of the larva is also observed and it is 5·2 mm. which is 21·5 times in length.

The head is moderately long and measures 4 mm. which is 28 times in length. A slight increase in the length of the snout is observed and it is 2·9 times in head. The proportion of the eye has also increased to 4·4 times in head. The nostrils have begun to differentiate in this stage and the anterior one is situated midway between the tip of the snout and the front edge of the eye while the posterior one is in front of the eye. Three more teeth have been added to each half of the upper jaw increasing their number to 19 including the grasping tooth and the dental formula is 1 + 6 + 12/1 +12.

The type larva shows 144 myotomes and no change in the position of the anus has been observed in this stage and it is below the 65th myotome. Similarly the predorsal and preanal distances also do not show any change in their proportions to length.

The pectoral and caudal fins have become slightly longer than in the previous stage.

There is no change in the colouration of the larva. However, the ventral preanal and the mid-lateral postanal groups of chromatophores have become more prominent in this stage. All the chromatophores of the mid-lateral series including the anterior ones and those situated at the bases of the anal fin rays have assumed an elongated shape.

Stage I (Text-Fig. 4 and Photograph 1).—The leptocephalus which represents the final stage in the growth of the larva is characterised by its very long and compressed body which gradually tapers towards the head and the tail. There is very little increase in the height of the larva when compared with the previous stage and it measures 5·3 mm. which is 24·2 times in length.

The head which measures 4·4 mm. in length is relatively small when compared with the body and it is 29·1 times in length. The eye does not show any increase in its diameter, however, its proportion in head has increased to 4·9 times. The snout has become slightly longer but continues to be 2·9 times in head. The nostrils have become clearly differentiated and

there is no change in their relative positions from the condition found in the previous stage. The teeth do not show any change in their number and arrangement and the dental formula of the leptocephalus is the same as that of the preceding stage.

The type specimen shows 146 myotomes with the anal opening below the 67th myotome. The proportion of the preanal distance in length is 1·8 which is slightly less than that of the previous stage while the predorsal distance continues to be 1·1 in length. The position of the vent is observed to be fairly constant in all the specimens, even though the number of myotomes varies from 144 to 147.

There is no change in the general pigmentation of the leptocephalus. The chromatophores on the head have become somewhat diffuse and a few more pigment cells have appeared in the heart region.

REMARKS

The four stages described above clearly show the changes undergone during the growth of the larva into the full-grown leptocephalus. There is considerable increase in the total length of the different stages while the height does not register proportionate increase and consequently shows decreasing percentage values as is evident from Table I. On the contrary, the predorsal and preanal distances show progressive increase which is proportionate to that of the length of the larva and on account of this uniform increase their percentage values are more or less constant in the 4 stages. It may be pointed out here, that in the growing phase of the leptocephalus of *Muræsox talabonoides* (Nair and Mohamed, 1960) both these distances show marked decrease in the successive stages which is caused by the migration of the anus and the origin of the dorsal fin even before the larva reaches the full growth.

The head also grows longer in the different stages, but the growth is not proportionate to that of body and, therefore, a reduction is seen in the percentages. Similarly the snout also does not show proportionate increase in length to that of the head and consequently its percentage also decreases in the successive stages. The opposite condition is found in *M. talabonoides* where the snout shows considerable increase in the different growing stages. The diameter of the eye also does not show any increase in most of the stages and consequently its value decreases in the different stages due to the lengthening of the head. Another feature noticed during the growth of the larva is the addition of new teeth in the second group of the upper jaw; the total number increasing from 15 to 19 in each half while the lower jaw shows

only the same number of 13 teeth in all the stages. In *M. talaboroides* also an additional tooth is formed during the growing phase.

The vent of this larva does not show any significant change in its position in relation to the myotomes during growth and in this respect differs from the condition found in *M. talabonoides* where the anterior migration of the anus takes place even before reaching the full growth of the leptocephalus.

Among the ophichthyid leptocephali recorded so far from Indian waters, Leptocephalus I is interesting in showing the maximum length and minimum height as seen from Table II which gives the characters of all the Indian leptocephali of the family Ophichthyidæ. In spite of its unusual length, it shows only 146 myotomes with the vent below the 67th muscle segment. The only known ophichthyid leptocephalus showing a very similar condition is Leptocephalus II collected by Jones and Pantulu (1955) from the Travancore coast. This larva shows 141 myotomes with the vent below the 66th myotome. A close similarity is also noticed in the length of the head, predorsal and preanal distances and also in the size of the eye. The length of the snout of the Travancore larva given by the authors is only 0·53 mm. whereas in the description they state that the "snout is twice as long as the orbit" and the diameter of the latter is given as 0·78 mm. Obviously there is an error in the measurement of the snout given in the table and if it is twice the diameter of the eye, then the percentage of the snout length of this larva is found to be fairly close to that of the Bombay larva.

Even though there is general agreement in regard to myotome number and body proportions, it is seen that the height of the Travancore larva is nearly twice that of the Bombay larva. Difference is also noticed in the dentition of the two larvæ. Jones and Pantulu state that "in addition to the prominent median tooth originating a little above the tip of the upper jaw there are 15 pointed teeth on each side" and their reference to a median tooth is not clear. It is likely that they are referring to one of the paired grasping tooth, the other being missing in the single specimen collected by them. On the basis of this assumption, the Travancore specimen has 16/11 teeth while in the Bombay specimen 19/13 teeth are present.

Apart from these minor differences, the most important one is in the colouration. In the Bombay larva 7 groups of chromatophores are found in the alimentary canal in the 6 hump-like swellings and the last one near the anal opening and in addition to these, 6 groups of postanal mid-lateral chromatophores are also present. Travancore leptocephalus also shows pigment cell concentrations on the hump-like swellings, but their number

TABLE II

Measurements of ophichthyid leptocephali

	Leptocephalus vermicularis Southwell and Prashad, 1919	Larva VI Deraniyagala, 1934	Leptocephalus A Gopinath, 1950	Leptocephalus C Gopinath, 1950	Leptocephalus F Gopinath, 1950	Leptocephalus (Madras) Jones and Pantulu, 1955	Leptocephalus I (Travancore) Jones and Pantulu, 1955	Leptocephalus II (Travancore) Jones and Pantulu, 1955	Leptocephalus of *Pisoodonophis hijala* Jones and Pantulu, 1955	Leptocephalus I	Leptocephalus II	Leptocephalus III
Total length ..	61·2	90·0	99·0	82·0	104·0	105·0	82·0	103·5	68·0	128·0	96·0	72·0
Maximum height ..	4·1 (6·7)	8·0 (8·9)	8·5 (8·6)	7·0 (8·5)	7·5 (7·2)	6·3 (6·0)	6·4 (7·8)	7·3 (7·1)	5·1 (7·5)	5·3 (4·1)	7·0 (7·3)	5·0 (6·9)
Predorsal distance ..	44·8* (73·2)	21·7* (24·1)	..	..	..	97·8 (93·1)	77·0 (93·9)	97·1 (93·8)	61·3 (90·1)	119·6 (93·4)	87·2 (90·8)	68·9 (95·7)
Preanal distance ..	23·4† (38·2)	50·0* (55·6)	47·0 (47·5)	34·0 (41·5)	43·5 (41·8)	40·0 (38·1)	54·0 (65·9)	52·5 (50·7)	28·0 (41·2)	69·2 (54·1)	53·4 (55·6)	37·3 (51·8)
Length of head ..	4·3 (7·0)	4·7† (5·2)	4·5 (4·5)	3·4 (4·1)	3·5 (3·4)	4·1 (3·9)	2·9 (3·5)	3·8 (3·7)	3·4 (5·0)	4·4 (3·4)	5·4 (5·6)	3·3 (4·6)
Length of snout ..	1·1 (25·6)	..	..	..	..	1·3 (31·7)	1·0 (34·5)	1·5 (39·5)	1·0 (29·4)	1·5 (34·1)	1·4 (25·9)	1·0 (30·3)
Diameter of eye ..	0·6 (14·0)	..	..	..	..	0·5 (12·2)	0·6 (20·7)	0·8 (21·1)	0·5 (14·7)	0·9 (20·5)	1·1 (20·4)	0·6 (18·2)
Total myotomes ..	122	158	156	206	183	223	174	141	155	146	146	157
Preanal myotomes ..	47	70	63	79	66	80	107	67	60	67	67	65
Teeth ..	..	5/5	22/20	10/14	24/22	12/10	14/10	16/11	nil	19/13	19/13	12/10

* Calculated from the Text-Figures. † Calculated from the description.

is not given by the authors. However, the post-anal mid-lateral groups of chromatophores are absent in the Travancore larva. This difference in the pattern of pigmentation, taken in conjunction with the other differences, indicates that the two larvæ belong to different species.

LEPTOCEPHALUS II

(Text-Fig. 5 and Photograph 2)

Total length	..	96·0
Maximum height	..	7·0 (7·3)
Predorsal distance	..	87·2 (90·8)
Preanal distance	..	53·4 (55·6)
Length of head	..	5·4 (5·6)
Length of snout	..	1·4 (25·9)
Diameter of eye	..	1·1 (20·4)
Total myotomes	..	146
Preanal myotomes	..	67

This leptocephalus which also belongs to the family Ophichthyidæ is not very common in Bombay waters and the collection contains 14 specimens (*vide* Introduction, Nair and Mohamed, 1960). This larva shows a striking resemblance to Leptocephalus I in general appearance, myotome number and pigmentation and at first was thought to belong to the same species, but a critical study showed that the two larvæ differed both in morphometric and meristic characters and it was, therefore, thought desirable to describe it separately, in view of the possibility that it may belong to a very closely related species.

The leptocephalus is completely opaque and is much thicker than the previous larva. It is 96 mm. in length with a more or less uniform height in the postanal region. The anterior portion tapers gradually towards the head while the caudal end tapers abruptly and terminates in a pointed free tail. The height is 7 mm. which is greater than that of Leptocephalus I and it is only 13·7 times in length.

The head is moderately long and measures 5·4 mm. and it is 17·8 times in length. The snout is conical, pointed and very short and is contained 3·9 times in head. The eye is larger than that of Leptocephalus I and it is 4·9 times in head. The nostrils are clearly differentiated and the anterior one is situated midway between the tip of the snout and the front edge of the eye

while the posterior nostril is immediately in front of the eye. The jaws are of the same length and the gape of the mouth extends to a level with the centre of the eye. Both the jaws are provided with forwardly directed pointed teeth and the dental formula is $1 + 6 + 12/1 + 12$. Each half of the upper jaw carries in addition to the curved grasping tooth, 6 long teeth which become gradually smaller backwards and 12 uniformly sized, regularly arranged short teeth. The lower jaw carries in each half a grasping tooth and 12 teeth of which the anteriormost one is the longest while the succeeding ones become shorter with the last one the smallest in the series.

Most of the larvæ possess 146 myotomes while a few larvæ showed 145 muscle segments. The alimentary canal which is of uniform thickness takes a straight course with the vent opening to the outside below the 67th myotome and shows 6 ganglion-like swellings at regular intervals in all the larvæ in the collection. This obviously shows that there is no shifting of the anus during the growth of the leptocephalus. The preanal distance is 1·8 in length while the predorsal distance which is very long is 1·1 in length.

The pectoral fin is very small with indistinct rays. The anal fin shows only 154 rays which is considerably lower than the count in Leptocephalus I. The dorsal fin rays show variation in their number owing to the growth of the fin and formation of new rays in the different specimens present in the collection. The caudal fin is absent in the larva.

The head pigmentation is very simple and along the border of the middle portion of the upper jaw a few small chromatophores are present in a line. A small group of 3–4 large, dendritic chromatophores is present in front of the heart.

The mid-lateral row of chromatophores commences from the 9th myotome and all the succeeding myotomes are provided with a highly elongated black chromatophore with a few exceptions here and there. In the post-anal region 6 prominent mid-lateral groups of pigment cells are clearly seen and these are situated at intervals of 10–12 myotomes. These are formed by the elongation of two consecutive pigment cells of the mid-lateral series and also by the presence of an internal group of chromatophores below the vertebral column. Chromatophores are also concentrated in the form of black patches on the dorsal side of the alimentary canal and altogether 7 such groups are present arranged equidistant from one another. The first six patches are on the ganglion-like swellings of the alimentary canal while the last one is near the anal opening. In some larvæ 2–3 smaller groups of chromatophores have been observed to be present in between some of the posteriormost pigment groups of the alimentary canal. Variations in the

number of pigment cells in each small group as well as in the number and arrangement of these groups have been frequently seen.

The anal fin is provided with a row of long diffuse chromatophores at the base of each of the rays excepting a few of the anteriormost rays. The dorsal fin is free from pigment cells and sometimes one or two dendritic chromatophores are seen on the dorsal side of the caudal extremity of the vertebral column.

Remarks

As pointed out earlier, this larva bears a striking resemblance to Leptocephalus I in the size of the eye, dental formula, myotome number, position of the vent and the predorsal and preanal distances. The myotome number varies in this larva from 145 to 146 only while in Leptocephalus I a higher variation from 144 to 147 is seen. However, the important differences are in its shorter length and greater height of which the latter is nearly 1¾ times that of Leptocephalus I. Further, the head of this larva is longer and is about 1⅓ times that of Leptocephalus I. These characters which have been found to be stable are very helpful in separating the 2 species.

Among the meristic characters, the anal fin alone is taken into consideration since the dorsal fin is observed to show variation in the fin ray count depending on the stage of growth of the larva. The present larva shows a count of 154 anal fin rays only while a higher count of 186 rays is present in the anal fin of Leptocephalus I. It may be mentioned here that Lea (1913) while discussing the importance of myotome number in referring leptocephali to their adults says that " different species of eels have about the same number of segments (vertebræ) and that the number of segments in different individuals of the same species may vary " and, therefore, it is " necessary to include other characters such as the number of rays". In spite of the close similarity, the few differences in the morphometric and meristic characters indicate the possibility that this leptocephalus may belong to a very closely related species or at least to a variety of the same species.

Leptocephalus III

(Text-Fig. 6)

Total length	..	72·0
Maximum height	..	5·0 (6·9)
Predorsal distance	..	68·9 (95·7)
Preanal distance	..	37·3 (51·8)

Length of head	..	3·3 (4·6)
Length of snout	..	1·0 (30·3)
Diameter of eye	..	0·6 (18·2)
Total myotomes	..	157
Preanal myotomes	..	65

This ophichthyid leptocephalus is represented in the collection by a single specimen which was obtained from the plankton collection made off Bombay on the 1st May 1953 (*vide* Introduction, Nair and Mohamed, 1960). The larva was alive when the collection was brought to the laboratory, but owing to its extreme rarity it was thought best to preserve it rather than allowing it to metamorphose in the laboratory.

The larva is transparent and measures 72·0 mm. in length. The height is more or less uniform barring the extremities which gradually taper, the posterior of which terminates in a blunt tail. The height of the larva is 5 mm. and it is 14·4 times in length.

The head is very small and measures 3·3 mm. and is contained 21·8 times in length. The eye which measures only 0·6 mm. in diameter is small in comparison with that of the other two ophichthyid leptocephali described here, and it is 5·5 times in head. The nostrils have become clearly differentiated and the anterior one is situated in the middle of the snout while the posterior one is midway between the anterior nostril and the front margin of the eye. The cleft of the mouth is straight and extends a little beyond the centre of the eye. The jaws are of equal length and are provided with pointed forwardly directed teeth and the dental formula is $1 + 4 + 7/? + 9$. Each half of the upper jaw carries a strongly curved and pointed grasping tooth followed by 4 strong and long teeth which become shorter backwards. This group is followed by another set of 7 minute conical teeth. The grasping tooth of the lower jaw is missing while the other teeth are intact and each half of the jaw is provided with 9 teeth of which the anteriormost one is the longest while the succeeding ones become smaller with the last one the smallest in the series.

There are 157 distinct myotomes in the larva and the anus is below the 65th myotome. The alimentary canal is somewhat short and does not extend up to the middle of the larva. It becomes wider posteriorly and it is looped in a characteristic festoon-like manner with 7 sections. The 6 angular portions of the loops are below myotomes 12–13, 20–21, 29–30, 37–38, 46–47 and 54–55. The preanal distance is 1·9 in length while the predorsal distance is nearly 1 owing to the extremely short nature of the dorsal fin.

The pectoral fin is in the form of a small circular flap-like structure without rays. Both the dorsal and anal fins show distinct rays and their number is 46 and 284 respectively. Some of the posteriormost rays, excepting the last few ones, of the dorsal and anal fins are very long and show a beautiful pattern of uniform zig-zag curvature (Photograph 3).

Head pigmentation is very feeble and consists of 3 small pigment cells situated below the nostrils very near the border of the upper jaw and no chromatophores are present in the heart region.

The mid-lateral pigmentation is not very conspicuous and the chromatophores which commence from the 7th myotome are arranged just below the vertebral column. A few of the anteriormost myotomes are provided with only a single round pigment cell while the rest are usually provided with 3 elongated highly branching chromatophores. These chromatophores are arranged end to end and appear as a dark line along the myocommas.

The pigmentation of the alimentary canal is characteristic and a cluster of highly branching black chromatophores is present above the 6 hump-like thickened angles of the loops of the alimentary canal. A similar concentration of pigment cells is present on the dorsal side of the extremity of the alimentary canal near the anal opening. A few chromatophores are also present on the ventral side of the first 4 hump-like thickenings of the alimentary canal. These dark patches at the corners of the loops tend to enhance the festoon-like appearance of the alimentary canal. Four to five unbranched pigment cells are arranged in a line on the ventral side of the first two curved sections of the alimentary canal.

The pectoral and dorsal fins are free from pigment cells while 2–3 chromatophores are present at the base of each of the rays of the anal fin.

Remarks

Quite a few ophichthyid leptocephali have been recorded from Indian waters, but those possessing looped alimentary canal are few in number. Deraniyagala (1934) collected a preleptocephaline larva off Cape Comorin (Larva V) measuring 20 mm. in length and possessing 119 myotomes of which 99 are preanal in position. The figure of the larva shows clearly 9 loops in the alimentary canal. Larva II collected off Tuticorin by Nair and Bhimachar (1950) is also a preleptocephaline stage showing a characteristic pigmentation and the formation of 6-looped portions in the alimentary canal. This larva measures only 7 mm. in length but shows a high myotome number of 180 of which 78 are preanal in position. These preleptocephaline arvæ, which have been referred to the family Ophichthyidæ by Nair (1960),

and Nair and Dharmamba (1960) respectively, represent very early larval stages in the growth and as such their morphometric characters are not strictly comparable with those of the fully grown leptocephalus and, therefore, they have not been taken into consideration here.

Deraniyagala (1934) recorded another ophichthyid leptocephalus (Larva VI) with looped alimentary canal and in spite of the brief description, the figure shows clearly the looped nature of the alimentary canal with 7 sections in it. This larva possesses 158 myotomes of which 70 are preanal in position. The general characters and pigmentation of the larva have not been given by him. However, from the figure it is seen that the origin of the dorsal fin in this larva is far in front and it is " closer to the snout tiq than to the cloaca ", indicating that the dorsal fin is long, in contrast to the short nature of this fin in the Bombay larva and normally found in the other ophichthyid leptocephali. It is seen that the larva has begun to metamorphose as shown by the absence of the larval set of teeth and the formation of the adult set which is indicated by " 5 short caninoid teeth on each jaw ". It is, therefore, possible that with the commencement of metamorphosis, the dorsal fin develops rapidly and assumes the normal size found in the adult.

Gopinath (1950) recorded another ophichthyid larva (Leptocephalus) A with looped alimentary canal which shows 156 myotomes with the vent below the 63rd myotome. The larva shows 8 distinct loops with pigment concentrations over the ganglion-like swellings at the angles between the two successive loops and in addition shows 9–10 postanal mid-lateral groups of chromatophores.

The larva described in this paper shows a striking similarity to those described by Deraniyagala (1934) and Gopinath (1950) especially in the myotome number which is 157, 158 and 156 respectively. The position of the vent shows some variation and it is below the 70th myotome in the Ceylon larva while it is below the 63rd and 65th myotomes in the Trivandrum and Bombay larvæ respectively. These 3 larvæ also differ in the number of loops found in the alimentary canal and the Trivandrum larva shows 8 loops while only 7 loops are present in the Ceylon and Bombay larvæ.

A comparison of the colouration of these larvæ is not possible since there is no mention about the pigmentation of the Ceylon larva. The Trivandrum larva shows a characteristic pigmentation and chromatophore concentrations are present in the ganglion-like swellings at the angles of the loops and also on the sides of the postanal portion of the body very much resembling the pigmentation of Leptocephalus I and II described earlier. The Bombay larva also shows similar accumulation of pigment cells at the

angles of the loops of the alimentary canal, but the postanal mid-lateral black patches are not present in this larva.

In spite of the close similarity in the total and preanal myotomes between these 3 larvæ recorded from the coastal waters, the Trivandrum larva is distinct in showing 8 loops in the alimentary canal and also in the characteristic postanal pigmentation. The Ceylon larva and the present one show similarity in the number of loops and differ only in minor details like height, length of head, predorsal distance, etc. A comparison of the colouration is not possible without a re-examination of the Ceylon larva. If a similar colouration is present in the Ceylon larva also, then it is highly probable that they belong to the same species.

Leptocephalus vermicularis (Southwell and Prashad, 1919) collected from Doorakara, Sunderbans (Gangetic Delta) is the first ophichthyid leptocephalus recorded from Indian waters (Nair, 1960). Deraniyagala (1934) described 2 larvæ, Larva V and Larva VI and he referred the latter only to the family Ophichthyidæ due to the absence of the caudal fin. The ophichthyid characters of Larva V (Deraniyagala, 1934) and Larva II (Nair and Bhimachar, 1950) have been pointed out by Nair (1960) and Nair and Dharmamba (1960) respectively. Gopinath (1950) described Leptocephalus A and Leptocephalus C collected from the Trivandrum coast and assigned them to the ophichthyid group. Leptocephalus F described by him is also an ophichthyid larva (Nair, 1960). Jones and Pantulu (1955) described a few ophichthyid preleptocephaline stages and 4 ophichthyid leptocephali collected from Madras, Travancore and Orissa coasts and referred the last one to *Pisoodonophis hijala* based on the myotome and vertebral counts. Recently Nair and Dharmamba described the early life-history of an ophichthyid egg collected from the Lawsons' Bay, Waltair (1960).

Including the 3 larvæ described here, 16 ophichthyid leptocephali have so far been recorded from Indian waters of which 4 are in the early preleptocephaline stages. The important characters of all the leptocephali are given in Table II which shows clearly the wide variation encountered among the different larvæ in regard to size, morphometric characters, myotome number and to some extent in pigmentation.

As pointed out earlier, Leptocephalus I described here is peculiar in showing the maximum length of 128 mm. and minimum height of 4·1% while the minimum length of 61·2 mm. and maximum height of 8·9% are seen in *Leptocephalus vermicularis* and Larva VI (Deraniyagala, 1934) respectively.

The length of head and snout and the diameter of eye do not show any significant difference among the different ophichthyid leptocephali. Leptocephalus I and II collected from Bombay show the maximum number of teeth namely 19/13. Deraniyagala (1934) mentions the presence of the lowest number of " 5 short caninoid teeth on each jaw " in Larva VI, but they appear to be the rudiments of the growing adult set of teeth only. Gopinath (1950) gives 22/20 and 24/22 teeth for Leptocephalus A and F which obviously represent the total number of teeth present in each jaw and not the number found in each half of the jaw, the latter of which is the customary way of expressing the number of teeth in leptocephali. This assumption is found to be correct in the case of *Muraenesox cinereus* for which he gives 32/30 teeth while each half of the jaws carries 15/15 as shown in the figure (Nair, 1947). It is believed that he has followed the same procedure in regard to the number of teeth in the other larvæ also and Leptocephalus C described by him shows a low number of 5/7 (10/14 as given by Gopinath) which as pointed out by him, does not represent the full set of larval teeth. In regard to Leptocephalus II from Travancore coast Jones and Pantulu (1955) mentions the presence of a prominent median tooth which as pointed out earlier could only be one of the paired grasping tooth.

The myotome number shows high variation among the different leptocephali and in this respect the larva described by Jones and Pantulu (1955) from Madras shows the highest number of 223 myotomes while *L. vermicularis* shows the lowest number of 122 myotomes. It is seen that the predorsal distance in most of the species is more than 90% of the length except in *L. vermicularis* and Larva VI of Deraniyagala (1934) where the lower percentage is caused by the elongation of the dorsal fin due to the commencement of metamorphosis. On the contrary, the preanal distance does not show much variation among the different ophichthyid leptocephali. The position of the vent shows considerable divergence and it is below the 107th myotome in Leptocephalus I collected from Travancore coast (Jones and Pantulu, 1955) while it is below the 47th myotome in *L. vermicularis*.

The alimentary canal of most of the ophichthyid larvæ is straight except in Larva VI of Deraniyagala (1934), Leptocephalus A of Gopinath (1950) and Leptocephalus III from Bombay in which it shows a looped nature. The gut of the other larvæ shows hump-like thickenings at regular intervals. There is no information on this point about the alimentary canal of *L. vermicularis* (Southwell and Prashad, 1919) and Larva VI of Deraniyagala (1934), the descriptions of which are very brief. Gopinath (1950) recorded 8–9 and 6 distinct humps for Leptocephali C and F respectively. A similar condition has been noted by Jones and Pantulu (1955) in all the ophichthyid

larvæ described by them. They have recorded the presence of 6 and 12 hump-like thickenings for the leptocephalus of *Pisoodonophis hijala* and the Leptocephalus from Madras respectively while they mention the presence of similar thickenings in the alimentary canal of the other two larvæ collected from Travancore coast without giving their number. Leptocephali I and II from Bombay waters also show this character distinctly and 6 such thickenings are present in both the larvæ. Such hump-like thickenings of the alimentary canal have also been noticed in the leptocephali with looped alimentary canal and they are found at the angles of the loops. It may be pointed out here that such hump-like thickenings of the alimentary canal have so far been recorded only in the ophichthyid leptocephali and as such this feature can be regarded as a diagnostic character of the larvæ of this family.

The different ophichthyid leptocephali show a fair amount of uniformity in regard to pigmentation with slight variations which perhaps indicate specific differences. From the available information, it is seen that in almost all the larvæ, the head pigmentation is normal and consists of a group of chromatophores in the middle portion of the upper jaw and in the region of the heart. The mid-lateral row of chromatophores is distinctly seen in all the larvæ below the vertebral column with only slight variations in the arrangement of the pigment cells. In all the larvæ, the anal fin is pigmented and the chromatophores are found at the bases of the fin rays. The dorsal fin, on the other hand, is unpigmented in all the leptocephali except in Leptocephalus F of Gopinath (1950). The most important feature of the pigmentation is the concentration of pigment cells in the form of dark patches in the hump-like thickenings of the alimentary canal and also occasionally in between them. A similar conspicuous group of chromatophores is also present in some larvæ near the anal opening. In addition to this pigmentation, Leptocephalus A of Gopinath (1950) and Leptocephali I and II from Bombay waters show additional conspicuous postanal groups of chromatophores situated internally at regular intervals below the vertebral column.

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EXPLANATION OF PLATE

PHOTOGRAPH 1. Leptocephalus I, × *ca.* ¾.

PHOTOGRAPH 2. Leptocephalus II. About natural size.

PHOTOGRAPH 3. Caudal region of Leptocephalus III showing the zig-zag curvature of the dorsal and anal fin rays.

PHOTO. 1

PHOTO. 2

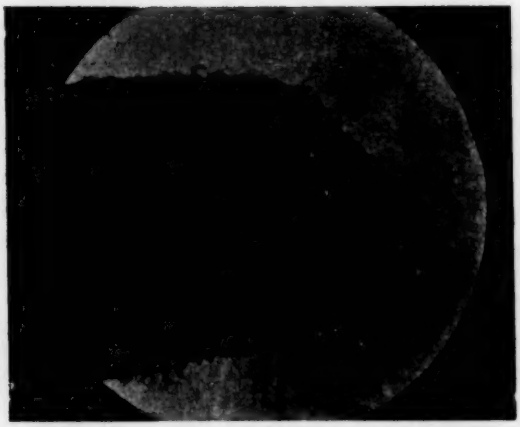

PHOTO. 3

STUDIES ON THE LEPTOCEPHALI OF BOMBAY WATERS

V. A Few Other Leptocephali*

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Leptocephalus IV

(Text-Fig. 1 and Photograph 1)

Total length	..	68·5
Maximum height	..	10·3 (15·0)
Predorsal distance	..	39·0 (56·9)
Preanal distance	..	50·5 (73·7)
Length of head	..	4·3 (6·3)
Length of snout	..	1·3 (30·2)
Diameter of eye	..	1·2 (27·9)
Total myotomes	..	164
Preanal myotomes	..	105

A single specimen of this murænid leptocephalus was obtained from the 'dol' net catches made at Versova on the 9th Januray 1956 (*vide* Introduction, Nair and Mohamed, 1960 *a*).

The larva is transluscent and short and measures only 68·5 mm. in total length. The body is moderately thick and very high and shows the typical leaf-like appearance. The maximum height of 10·3 mm. is slightly in front of the anus and it is 6·7 times in length. The body tapers moderately at the extremities and terminates posteriorly in a rounded caudal fin.

The head which measures 4·3 mm. is 15·9 times in length. It is roughly conical in shape with a blunt snout which is 3·3 times in head. The eye is

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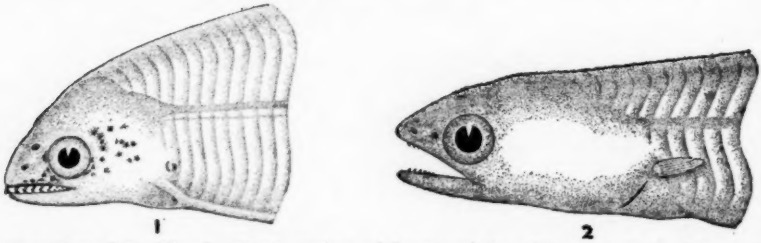
perfectly circular and it is 3·6 times in head. Both the jaws are of equal length and the cleft of the mouth extends to a level with the centre of the eye. Jaws are provided with strong and pointed teeth and the dental formula is 1 + 5 + 3/1 + 5 + 4. Each half of the upper jaw carries a short grasping tooth, 5 strong and pointed teeth which gradually decrease in length backwards and another set of 3 short, conical and uniformly sized teeth. The lower jaw also carries in each half, teeth of the same size, shape and arrangement but they number 10 since an additional tooth is present in the last group. The nostrils are distinctly seen and the anterior one is midway between the tip of the snout and the front border of the eye while the posterior one is above and slightly behind the anterior nostril.



TEXT-FIGS. 1–2. Fig. 1. Head region of Leptocephalus IV, ×*ca.* 5·5. Fig. 2. Head region of Leptocephalus V, ×*ca.* 6.

The myotomes are very narrow and compactly arranged. There are 164 myotomes in the larva and a distinct diaphanous zone where the myotomes do not extend is present on the dorsal side throughout the length of the leptocephalus. A similar zone is seen on the ventral side also, but it begins only from the middle of the larva and extends up to the tail. These transparent zones are relatively wide in the caudal region. The dorsal fin originates slightly behind the middle of the larva opposite the 77th myotome. The predorsal distance is 1·8 times in length. The vent is below the 105th myotome and the preanal distance is 1·4 times in length.

The dorsal and anal fins are continuous with the caudal fin and the posteriormost rays of these two fins are double the length of the other rays and equal in length to those of the caudal fin. The rudiments of the pectoral fin is seen as a frilled fold near the gill opening.

The colouration of this larva differs from that of the other murænid larvæ recorded so far from Indian waters and it is conspicuously pigmented in the head region and it serves as a distinguishing feature of the leptocephalus. Head pigmentation of diagnostic importance is rare in

Indian leptocephalids and so far it has been observed only in the case of *Uroconger lepturus* (Nair, 1946) and in a few other murænid leptocephali (Nair, 1947 and 1948; Jones and Pantulu, 1952). A group of large highly branching chromatophores is present in the snout in the form of a dark patch extending from the middle of the snout along the border of the upper jaw up to the front edge of the eye. A large group of several similar pigment cells occurs behind the eye. The roof of the mouth is also black owing to the dense accumulation of black branching chromatophores. These patches of chromatophores on either side of the eye together with the buccal pigmentation give a conspicuous dark colouration to the head of the leptocephalus.

A row of small unbranched pigment cells is seen on the dorsal side of the posterior half of the alimentary canal and these chromatophores are regularly arranged in the post-aortic region.

A row of closely arranged small black chromatophores is seen at the base of the anal fin and the pigment cells are generally found at the base of the fin rays. Dorsal fin shows a row of black pigment cells, but they are arranged far apart tending to become closer in the caudal region. In front of the dorsal fin minute chromatophores are present along the mid-dorsal line and this row extends anteriorly up to the middle of the predorsal distance. A few scattered black chromatophores are present at the base of the caudal fin.

Remarks

Leptocephali belonging to the family Murænidæ are very common in Indian coastal waters as judged by the relatively large number of varieties recorded so far. Including the present form 14 varieties of murænid leptocephali and 1 murænid preleptocephaline stage have been recorded from both the coasts of India. *Leptocephalus dussumieri* described by Kaup (1856), *Leptocephalus milnei* recorded by Southwell and Prashad (1919) from the estuaries of the Gangetic Delta and Leptocephalus E collected by Gopinath (1950) from the Trivandrum coast have been identified as murænid leptocephali (Nair, 1960). Kaup's description of *L. dussumieri* is based on 3 specimens of which one was sent by Dussumier from Malabar and according to Bertin (1935) this specimen is not found in the Paris Museum. However, for comparison the additional characters given by Bertin in his re-description of the species are also included in Table I which gives the salient features of all the murænid leptocephali recorded so far from the Indian waters. Larva I collected by Nair and Bhimachar (1950) measures 7·7 mm.

TABLE I

Measurements of muræenid leptocephali

	Leptocephalus dussumieri (Kaup, 1856; Bertin, 1935)	*Leptocephalus milneri* (Southwell and Prashad, 1919)	Larva VIII (Deraniyagala 1934)	Larva IX (Deraniyagala, 1934)	Leptocephalus of *Muræna macrura* (Nair, 1947)	*Muræna* sp. (Nair, 1948)	Leptocephalus E (Gopinath, 1950)	Leptocephalus I (Jones and Pantulu, 1952)	Leptocephalus II (Jones and Pantulu, 1952)	Leptocephalus III (Jones andPantulu, 1952)	Leptocephalus of *Muræna tile* (Pantulu and Jones, 1954)	Muræna II (Pantulu and Jones, 1954)	Muræna III (Pantulu and Jones, 1954)	Leptocephalus IV
Total length ..	66·0	55·4	66·0	69·0	93·0	66·0	68·0	74·0	81·0	100·0	62·5	68·0	55·5	68·5
Maximum height ..	8·0 (12·1)	8·3 (15·0)	6·8 (10·3)	9·0 (13·0)	13·0 (14·0)	9·0 (13·6)	13·0 (19·1)	9·8‡ (13·2)	7·7‡ (9·5)	10·1‡ (10·1)	11·6 (18·6)	9 9 (14·6)	7·5 (13·5)	10·3 (15·0)
Predorsal distance ..	..	18·7* (33·8)	..	..	50·5 (53·8)	38·0 (57·6)	..	34·0 (45·9)	41·0 (50·6)	49·0 (49·0)	25·0 (40·0)	24·0 (35·3)	18·0 (32·4)	39·0 (56·9)
Preanal distance ..	..	42·2† (76·2)	38·9* (58·9)	39·6* (57·4)	62·0 (66·7)	47·0 (71·2)	46·0 (67·6)	50·0 (67·6)	56·0 (69·1)	63·0 (63·0)	51·5 (82·4)	57·0 (83·8)	45·5 (82·0)	50·5 (73·7)
Length of head ..	..	4·0 (7·2)	5·1† (7·7)	5·8† (8·4)	4·0 (4·3)	4·0 (6·1)	3·0 (4·4)	5·0 (6·8)	4·1 (5·1)	4·9 (4·9)	4·2 (6·7)	3·5 (5·1)	3·3 (5·9)	4·3 (6·3
Length of snout ..	..	1·3 (32·5)	..	..	1·2* (30·0)	1·5* (37·5)		0·9 (18·0)	1·1 (26·8)	1·3 (26·5)	1·0 (23·8)	0·9 (25·7)	0·7 (21·2)	1·3) (30·2)
Diameter of eye ..	..	1·1 (27·5)	..	..	0·9* (22·5)	1·0* (25·0)	..	0·5 (10·0)	1·0 (24·4)	0·9 (18·4)	0·9 (21·4)	0·9 (25·7)	0·9 (27·3)	1·2 (27·9)
Total myotomes ..	190	120	122	101	216	210	132	205	209	211	124	126	125	164
Preanal myotomes ..	110	79	69	55	120	126	79	116	123	119	97	98	93	105
Teeth ..	nil	?	nil	6/5	11/12*	nil	26/22	nil	nil	nil	12/10	17/12	17/12	9/10

* Calculated from the Text-Figures. † Calculated from the description. ‡ Excluding fins.

and possesses 146 myotomes of which 84 are preanal in position. This larva is referred to the murænid group by Nair and Dharmamba (1960), but it is not considered here since it is an early preleptocephaline stage.

Jones and Pantulu (1952) described 4 varieties of leptocephali and provisionally assigned Leptocephali I to III collected from the Burhabulong estuary in Orissa to the family Muræniдæ and doubtfully referred Leptocephalus IV which shows a striking resemblance to *Leptocephalus milnei* (Southwell and Prashad, 1919) and which was collected from the Hooghly estuary to *Muræna tile*. The identification of the leptocephalus of *M. tile* was later provisionally confirmed by them when they collected more murænid leptocephali from the Burhabulong estuary (Pantulu and Jones, 1954). They supplemented the description of the larva with an account of the stages in its metamorphosis and also described two more murænid leptocephali collected from the same place.

These authors have altogether described 6 larvæ murænid from the Burhabulong estuary out of the 14 recorded so far from Indian waters, but the diagnostic features or the significant differences between the larvæ are not given by them. A critical perusal of their papers shows that in addition to the myotome number, they have given importance to the body proportions also for creating the different varieties and consequently failed to recognise the presence of distinct species in the larval collection which showed a high variation from 180–216 myotomes. Further, they observed a constant myotome number in the leptocephalus and all the metamorphosing stages and the same vertebral number excluding the urostyle in the elver. It is well known that the myotome number is the chief character for comparing leptocephali and that the myotome and vertebral number varies in different individuals of the same species.

It is seen from Table I which gives the morphometric and other characters of all the murænid leptocephali recorded so far, that there is close similarity between some of the larvæ described by them in external characters, body proportions and myotome number. In Leptocephali II and III (Jones and Pantulu, 1952) the myotomes vary only by 2 segments which is only an intra-specific variation. According to their description, the height and head in both the larvæ are 10 and 20 times respectively in length, and there is almost perfect agreement in the other body proportions as well, which show that they belong to the same species. Leptocephalus I described by them differs from these two larvæ in myotome number, relatively higher body, very small size of the eye and snout and longer head.

In regard to the larva of *Muræna tile*, Muræna II and Muræna III, there is very close similarity in the myotome number as well as in body proportions (Table I) and the differences which they point out in these 3 larvæ are not of taxonomic significance. The difference in the myotome number by 2 muscle segments in *M. tile* and Muræna II and by 1 segment in Muræna II and Muræna III has, as pointed out earlier, no specific importance. The number of larval teeth is a variable character depending on the stage of growth of the leptocephalus. Referring to Muræna III they (1954) state that it "is distinct from the other two, described earlier in its characteristic notched constriction above the tip of the snout, the larger size of the eye in relation to that of the snout and in the number of the larval teeth". It may be pointed out here that the snout tends to become distorted due to improper fixation and such specimens are also present in the Bombay collection. In regard to the number of teeth, there is no difference between Muræna II and Muræna III and both have 17 teeth in the upper jaw and 12 teeth in the lower jaw, since the median tooth noted by them in Muræna II could only be one of the paired grasping tooth. It may also be pointed out here that all these murænid larvæ were collected from the same place on the same days. It is, therefore, necessary to consider both Muræna II and Muræna III as the larvæ of *Muræna tile* only.

Out of the 14 murænid larvæ recorded so far from Indian waters, only 11 are valid species. Leptocephalus III of Jones and Pantulu (1952) is considered synonymous with Leptocephalus II of the same authors while Muræna II and Muræna III (Pantulu and Jones, 1954) are only the leptocephalus of *Muræna tile* (Jones and Pantulu, 1952; Pantulu and Jones, 1954). A perusal of Table I which gives the salient features of all the murænid larvæ shows clearly the divergence in the characters seen among the different species. In regard to size, the longest larva is Leptocephalus II (Leptocephalus III) of Jones and Pantulu (1952) and it measures 100 mm. while *Leptocephalus milnei* (Southwell and Prashad, 1919) measures only 55·4 mm. in length. Compared with the leptocephalus of other families, those of Murænidæ possess the maximum height giving them the characteristic leaf-like appearance. Leptocephalus E of Gopinath (1950) shows the maximum height of 19·1% while the minimum height of 9·5% is recorded for Leptocephalus II of Jones and Pantulu (1952), the latter of which has begun to metamorphose as shown by the loss of the larval teeth.

In regard to the length of the head, marked difference is seen among these larvæ and the longest head of 8·4% is seen in Larva IX of Deraniyagala (1934) while the smallest head of 4·3% is seen in the leptocephalus of *Muræna macrura* (Nair, 1947).

The length of the snout and the diameter of the eye do not show any appreciable difference among the different murænid leptocephali except in the case of Leptocephalus I (Jones and Pantulu, 1952) where the eye is very small and about half the size of the eye of other larvæ. In regard to the number of teeth, the highest number is seen in *M. tile* with 17/12 teeth (Muræna II and Muræna III of Pantulu and Jones, 1954). As pointed out earlier (Nair and Mohamed, 1960 *b*), Gopinath's account of 26/22 teeth for Leptocephalus E could only be the total number of teeth in each jaw.

The predorsal distance which is more or less uniform in the different species shows only minor variations. The maximum predorsal distance of 57·6% is seen in *Muræna* sp. (Nair, 1948) while the minimum distance of 32·4% is seen in the leptocephalus of *M. tile* (Muræna III of Pantulu and Jones, 1954). On the other hand, the longest preanal distance is seen in *M. tile* (Muræna II of Pantulu and Jones, 1954) where it forms 83·8%. Larva IX of Deraniyagala (1934) shows the shortest preanal distance of 57·4%.

In regard to the myotome number, considerable difference is seen among the different larvæ and Leptocephalus II (Leptocephalus III) of Jones and Pantulu (1952) shows the maximum number of 211 muscle segments while the number is as low as 101 in Larva IX of Deraniyagala (1934). A similar variation in the number of preanal myotomes is seen among the different murænid leptocephali and Leptocephalus II (Jones and Pantulu, 1952) shows the maximum number of 123 preanal myotomes while Larva IX of Deraniyagala (1934) shows only 55 preanal myotomes.

It is well known that the adults of the family Murænidæ do not possess pectoral fins. But rudimentary fins have been observed to be present in a few forms like *Muræna macrura*, *M. tile*, etc. The pectoral fin atrophies and disappears during the process of metamorphosis as observed in the case of *M. macrura*.

The pigmentation of the murænid leptocephali shows certain distinctive features which are characteristic of the family. The head pigmentation has been observed to show variation in the different species and most of the murænid larvæ show the normal pigmentation while in some like Leptocephalus I (Jones and Pantulu, 1952) the head is completely free from pigment cells. The eye is distinctly pigmented in some larvæ like *M. macrura* and *Muræna* sp. (Nair, 1947 and 1948). In both the species the eye is golden yellow in colour with 8 large stellate black chromatophores arranged equidistant from one another round the pupil of the eye. In addition to this characteristic pigmentation of the eye, golden yellow patches are found in

the anterior and posterior regions of the eye of the leptocephalus of *M. macrura*. Similar conspicuous stellate chromatophores round the pupil of the eye have been observed by Jones and Pantulu (1952) in Leptocephalus II (Leptocephali II and III) which they thought " is not of any special diagnostic importance " since they disappear after preservation. The black pigment cells found in leptocephali do not disappear after fixation or even under long storage. It is likely that they become indistinguishable when the silvery colouration of the eye disappears during storage. The murænid larva collected from Bombay waters shows a distinctive colouration of the head in having black patches of pigment cells on either side of the eye closely resembling the pigmentation of the head of *Leptocephalus fuliginosus* (Stromman, 1896).

The body pigmentation is very faint in all the larvæ and the mid-lateral row of chromatophores is absent in the murænid larvæ which indicates that this is a family character. The alimentary canal shows pigmentation in a few larvæ only, like those of *M. macrura* and *M. tile*.

The anal fin is provided with chromatophores at the bases of the fin rays in all the leptocephali while the pigmentation of the dorsal fin is confined to the posterior region only as in the leptocephalus of *M. macrura*, Leptocephalus E of Gopinath (1950) and Leptocephalus I of Jones and Pantulu (1952).

LEPTOCEPHALUS V

(Text-Fig. 2 and Photograph 2)

Total length	..	83·5
Maximum height	..	5·2 (6·2)
Predorsal distance	..	33·0 (39·5)
Preanal distance	..	40·0 (47·9)
Length of head	..	6·2 (7·4)
Length of snout	..	1·3 (21·0)
Diameter of eye	..	1·4 (22·6)
Total myotomes	..	123
Preanal myotomes	..	51

A single specimen of this leptocephalus was collected from the 'dol' net catches landed at Sassoon Dock on the 21st April 1954 (*vide* Introduction, Nair and Mohamed, 1960 *a*). The larva is in a fairly advanced stage

of metamorphosis and it is described here in view of its extreme rarity in the Bombay waters.

The leptocephalus is transluscent, thick and measures 83·5 mm. in length. The height is almost uniform throughout its length except in the caudal portion which is bluntly pointed. The maximum height is 5·2 mm. which is 16·1 times in length.

The head is long and measures 6·2 mm. and it is 13·5 times in length. The snout is very short, obtusely pointed and much shorter than the diameter of the eye. The snout is 4·8 times in head. The eye is perfectly circular, large and measures 1·4 mm. in diameter which is 4·4 times in head. The nostrils are distinctly seen as small openings, and the posterior nostril is in front of the eye while the anterior one is midway between the tip of the snout and the posterior nostril. The cleft of the mouth is very short and extends up to the middle of the eye only. The larval set of teeth has fallen off and the permanent adult teeth are in the course of formation and they are seen clearly in the lower jaw, as short, pointed, inwardly directed teeth.

Due to the commencement of metamorphosis, the posterior myotomes are not clearly seen and about 123 muscle segments are present in the specimen. The alimentary canal is of moderate length and the vent opens to the outside below the 51st myotome and it is situated slightly in front of the middle of the larva with the preanal distance 2·1 times in length. The origin of the dorsal fin is in advance of the anus and is above the 42nd myotome with the predorsal distance 2·5 times in length.

The fins are well developed and the rays are distinctly seen in the anal and caudal fins while they are distinct only in the posterior portion of the dorsal fin. The pectoral fin is moderately long and pointed and shows indistinct rays. About 40 rays are present in the well-developed portion of the dorsal fin. The anteriormost rays of this fin are indistinct and could be made out only with difficulty. The posteriormost rays of the dorsal and anal fins, barring the last few ones, show a zig-zag curvature at their tips, similar to the condition observed in Leptocephalus III (Nair and Mohamed, 1960 *b*). The anal and caudal fins possess 110 and 6 distinct rays respectively.

The pigmentation of the leptocephalus is interesting and both the head and the body are completely devoid of chromatophores. The vertical fins, on the other hand, show somewhat prominent pigmentation and each of the fin rays of the anal and caudal fins and the well-developed posterior portion of the dorsal fin is pigmented at its base by a group of two to three

highly branching black chromatophores. These pigment cells surround the base of the rays to give a conspicuous colouration to the vertical fins.

REMARKS

This leptocephalus is completely different from any of the larvæ recorded so far from Indian waters and exhibits interesting peculiarities. The larva is in a fairly advanced stage of metamorphosis and judging from the morphometric characters, it corresponds to Stage III of *Muræsox cinereus* (Nair, 1947). The snout of the larva is very short and less than the diameter of the eye and in this respect approaches the condition found in some murænid leptocephali. The eye is very large and the larva possesses the largest eye so far observed in Indian leptocephali. The zig-zag curvature of the posteriormost rays of the dorsal and anal fins, excluding the last few ones, is similar to the condition found in the ophichthyid Leptocephalus III, but the presence of the well-developed caudal fin excludes it from the family Ophichthyidæ. The pigmentation also shows peculiarities and the head pigmentation normally found in other leptocephali is absent in this larva. Similarly, the mid-lateral pigmentation is also absent and in this respect resembles murænid larvæ. The pigmentation is confined to the base of the vertical fins only.

It was found difficult to assign this leptocephalus to any of the families of eels and the extreme rarity makes the identification very difficult and this can be attempted only at a later stage when more specimens become available for study.

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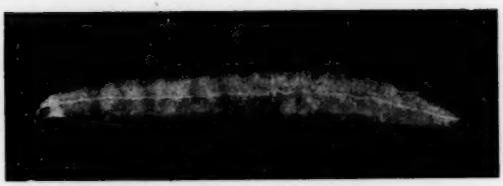

PHOTO. 1

PHOTO. 2

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Explanation of Plate

Photograph 1. Leptocephalus IV. About natural size.

Photograph 2. Leptocephalus V. About natural size.

ON THE EARLY DEVELOPMENT OF AN OPHICHTHYID EGG FROM THE LAWSON'S BAY, WALTAIR*

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Introduction

The eel eggs are relatively rare in the Indian coastal waters and consequently their occurrence has been recorded only on a few occasions. Aiyar, Unny and Varkey (1944) were the first to observe the presence of eel eggs in the plankton of the Madras coast, but since the full paper has not so far been published details regarding the eggs are not available. Nair and Bhimachar (1950) recorded the occurrence of a few eggs and early larvæ of the eels in the plankton off Tuticorin Bay, Gulf of Mannar. Their account deals with three varieties of eel eggs of which one was with an oil globule while the other two were without it. They also described two types of preleptocephaline larvæ which showed differences in the myotome number and pigmentation. Jones and Pantulu (1955) described three preleptocephaline larval stages obtained by rearing the eggs collected from the Madras plankton and as pointed out by them there is no evidence to show that the larvæ belong to the same type of egg. Bapat (1955) noted the presence of an eel egg without oil globule and which resembles Egg II, described by Nair and Bhimachar, in the plankton of Palk Bay and Gulf of Mannar and gave brief notes on the newly hatched larva obtained by rearing the eggs in the Laboratory.

The material for this study was obtained in the living condition from the regular plankton collections made from the Lawson's Bay, Waltair. The collections were made in the early morning hours with a bolting silk (No. 2) ¾ metre townet at a distance of about 3 miles from the shore where the depth is about 10 fathoms. This type of egg occurred in very small numbers in the townet collections made during the month of February, 1959, and they were reared in the Laboratory for obtaining the different stages described

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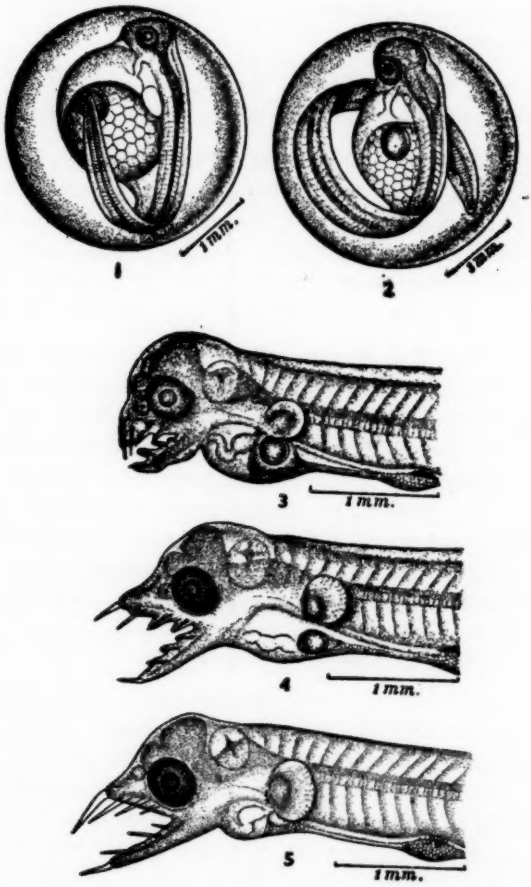
here. It was not possible to trace the larval development further owing to the paucity of the eggs in the collections.

Egg

The egg is transparent, large and perfectly spherical measuring 2·95 mm. in average diameter with a very wide perivitelline space. In the earliest



Text-Figs. 1–5. Fig. 1. Egg showing the earliest embryonic stage in the collection. Fig. 2. Egg showing the final embryonic stage just before hatching. Fig. 3. Head region on the one-day old larva. Fig. 4. Head region of the two-day old larva. Fig. 5. Head region of the three-day old larva.

developmental stage in the collection, the embryo is well formed with its posterior half free from the yolk mass (Text-Fig. 1). The yolk mass is clear, segmented and oval in shape. It is provided with a single yellow oil globule which is 0·4 mm. in diameter. A narrow posterior prolongation of the yolk mass extends on the ventral side up to the middle of the free portion of the embryo. The eyes, auditory vesicles and œsophageal pouch are well developed and the heart is functional in this stage. The myotomes have differentiated and several myotomes are present in the embryo. The larval finfold is seen distinctly and the embryo makes wriggling movements inside the egg membrane.

Minute brown chromatophores are present on the ventral side of the embryo behind the yolk mass and in its caudal region.

The final stage in the embryonic development just before hatching is shown in Text-Fig. 2. The embryo has grown considerably longer and lies curled up inside the egg membrane making frequent vigorous wriggling movements inside it. The yolk mass has become smaller with the oil globule situated in its anterior region. The backward prolongation of the yolk mass now extends more or less up to the posterior third of the embryo.

The pigmentation has become more defined and the pattern conforms to the ophichthyid pigmentation observed by Schmidt (1913) and Delsman (1933). Five groups of black ramifying chromatophores are present on the ventral preanal portion of the embryo behind the yolk mass and a network of similar chromatophores is also present on the caudal tip. The first two pigment groups are small while the others become larger and prominent posteriorly. The third group is situated at the tip of the prolongation of the yolk mass while the fifth group is near the developing vent. Minute black pigment cells have appeared in the posterior region of the eye.

ONE-DAY OLD LARVA

The larva after 24 hours, is perfectly transparent and measures 10·93 mm. in length with the maximum height in its middle region (Photograph 1). The posterior portion of the larva tapers gradually and terminates in the narrow rounded tail. The head is somewhat round in shape with the developing mouth subterminal in position (Text-Fig. 3). The jaws are very short and show the formation of the first set of larval teeth. The dental formula is $1+3/1+3$. The teeth are slender, pointed and covered by tissues and in both the jaws the grasping teeth are the longest while the others decrease in size backwards. The gape of the mouth extends to about the posterior third of the eye. The snout is very short and the nasal pit is situated in front

TABLE I

Measurements of the preleptocephaline larval stages

	One-day old larva	Two-day old larva	Three-day old larva
	mm.	mm.	mm.
Total length ..	10·93	11·57	12·36
Maximum height with finfolds ..	1·20	0·95	1·01
Maximum height without finfolds ..	0·78	0·76	0·76
Preanal distance ..	9·57	10·00	10·57
Length of head ..	0·86	0·95	1·05
Length of snout ..	0·27	0·38	0·34
Diameter of eye ..	0·34	0·36	0·38
Preanal myotomes ..	115	114	115
Total myotomes ..	202	203	205

of the eye and the latter is slightly oval in shape. The auditory vesicles have grown larger in size and are seen clearly. The yolk mass has become considerably smaller and its backward extension has broken up into four oval yolk pockets situated below the alimentary canal and also the first four groups of pigment cells. The myotomes are clearly seen especially in the preanal region and they become narrow and compactly arranged in the caudal region. The larva possesses about 202 myotomes of which 115 are preanal in position. The alimentary canal is very long, straight and the vent does not open to the outside. The pectoral fin has begun to develop and is seen as a flap-like structure. The finfold is continuous and begins from the nape and terminates on the ventral side at about the anterior third of the larva. The dorsal finfold is wide in the middle region of the larva.

There is no change in the pattern of pigmentation from that of the final embryonic stage. The head is unpigmented except the eye which is brown in colour due to the accumulation of brown chromatophores. One more ventral pigment group has formed in this stage and altogether 7 groups of branching brown chromatophores are present of which six are preanal in

position while the last one is at the tip of the tail. Each group is composed of several pigment cells. These groups are located above the alimentary canal and at the bases of myotomes 13–14, 23–24, 37–38, 53–56, 73–76 and 102–108. The post-anal group surrounds the caudal tip and almost covers it. In addition to these large groups three very small groups, each composed of 2–3 pigment cells, are also present in between the last four large groups of chromatophores. Of these small groups, two are preanal in position and are found on myotomes 64 and 88 while the third is post-anal and is on myotomes 123–124.

TWO-DAY OLD LARVA

The two-day old larva has increased slightly in length and measures 11·57 mm. A slight reduction in the height is seen and this is caused by the elongation of the larva and also by the slight decrease in the width of the finfold. However, the maximum height continues to be in the middle region of the larva. The head has grown longer than in the previous stage with well-developed mouth and jaws (Text-Fig. 4). The lower jaw is slightly longer than the upper. The dental formula continues to be the same and the teeth have become longer and are directed forwards. All the grasping teeth and the second pair of teeth of the upper jaw are exposed while the others are still covered by tissues. The snout has become pointed and the nasal pit is seen distinctly. The yolk mass has almost disappeared in this stage. The four yolk pockets are still seen distinctly though they are somewhat smaller in size. The oil globule has become smaller and it is covered by the remnants of the yolk mass. There is no change in the alimentary canal and the type specimen shows 114 preanal and 89 post-anal myotomes. The pectoral fin has grown larger in size. The anterior portion of the finfold has become very narrow and almost indistinguishable from the body.

The eye is black and partly silvery in colour. The pigment patches along the ventral side are more prominent and brownish-black in colour. These are situated on myotomes 13–14, 23–25, 35–36, 51–53, 71–75 and 101–104. The three small groups are on myotomes 62, 87 and 121–122. A few brown pigment cells have appeared at the base of the pectoral fin.

THREE-DAY OLD LARVA

The three-day old larva shows further increase in length and it measures 12·36 mm. The head has also grown longer with a pointed snout (Photograph 2 and Text-Fig. 5). The teeth in both the jaws have become longer and stronger than in the previous stage. The grasping tooth of the upper

jaw is very large and finely pointed while the others become smaller in size with the last one about one-fourth that of the grasping tooth. The teeth of the lower jaw are also long and pointed, but they are of about the same size. The yolk mass has completely disappeared while the oil globule is still seen even though it has lost its globular shape and is much smaller in size. The yolk pockets have also become smaller in size. The myotomes are distinct and about 205 myotomes are present in the type specimen with the vent situated below the 115th myotome.

The silvery eye and the ventral black pigment patches are very conspicuous in this stage. The ventral groups have assumed a linear shape and the preanal ones are situated on myotomes 13–14, 23–25, 36–38, 51–54, 76–80, and 102–106. The three small groups have also become prominent and are on myotomes 63–64, 88–90 and 123–25. In addition to these, one more small group has appeared in between the third and the fourth large group and it is on the 44th myotome. The chromatophores at the base of the pectoral fin have also become conspicuous by the formation of more pigment cells and these pigment patches on the ventral side give a characteristic dotted appearance to the larva.

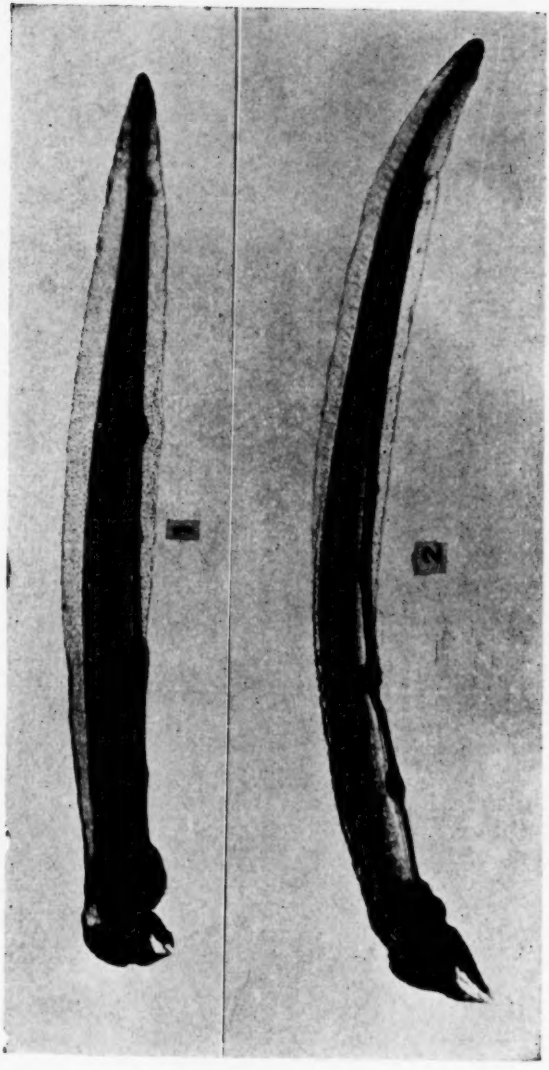
General Remarks

The identification of the eggs and preleptocephaline larval stages of eels is extremely difficult owing to the presence of the leptocephaline stage in the life-history of these fishes. The first successful attempt at identification by Schmidt (1913) was made possible only after getting the large 'Thor' collection of the transitional stages ranging from the tiny larvæ to the full grown leptocephalus. He found that the pigmentation and to some extent the number of myotomes are of great diagnostic value in identifying the youngest stages including the embryos. Based on the study of the 'Thor' collection made in the Mediterranean, Schmidt was able to identify the eggs and preleptocephaline larval stages of *Muræna helena*, *Nettastoma melanurum*, *Ophichthys serpens*, *Ophichthys hispanus* and *Ophichthys imberbis*. He found that certain features are common to the eggs and larvæ of the ophichthyid eels studied by him and stated that in the eggs of *O. serpens* and *O. hispanus* the yolk mass has a peculiar stalked shape and that most of the gut patches are developed even before hatching. He also pointed out that the pigmentation of the tiny larvæ is very characteristic in all the three ophichthyid larvæ in having a number of large conspicuous pigment groups along the gut, one or more such groups in the post-anal region and a well-marked patch at the end of the tail. Schmidt also noted that the swellings of the gut (liver, etc.) are common to these species. Delsman (1933) based on his study of the eel

eggs and larvæ of the Java Sea pointed out that such characteristically pigmented larvæ are hatched out from eggs with an oil globule.

The eggs and larvæ described in this paper show the general characters mentioned by Schmidt and Delsman for the Mediterranean and Java Sea ophichthyid species. The eggs show the typical stalked nature of the yolk mass and also most of the gut and caudal patches quite distinctly, especially in the later stages of embryonic development. Similarly the later larval stages described here show the post-anal patches also in addition to the gut and caudal patches. The gut swellings are not seen distinctly in the early larval stages and perhaps they are better developed in the later stages only. Specific identification of the eggs and larvæ is not possible in the absence of older larval stages and also information relating to the vertebral number of the common eels of the coastal waters, which are very essential for correlation.

Nair and Bhimachar (1950) described three types of eggs of which one showed the presence of an oil globule and in this respect resembles the egg described here. But the egg described by them is smaller in size and measures only 2·5 mm. in diameter and the figure of the egg shows the characteristic stalked nature of the yolk. It is, therefore, likely that it belongs to the ophichthyid group even though no indication of the pigmentation is seen in the developing embryo. Larva II described by them is an ophichthyid larva since it shows the characteristic pigmentation mentioned already. But it is difficult to decide whether the egg and larva belong to the same species since the embryo is not sufficiently developed to show the distinctive pigmentation. Egg II described by these authors apparently belong to another family since the yolk sac extends up to the anus and the anal fin terminates behind the vent unlike the condition found in the ophichthyid embryo and larva where it extends anteriorly far beyond the anus. Larva I described by them also shows a similar condition of the finfold. The straight disposition of the myotomes of the embryo and the larva, in contrast to the slightly angular nature of the myotomes of other larvæ, and their compact arrangement are generally seen in murænid larvæ only. It may be stated that these characters are also seen clearly in the photograph of the preleptocephaline stage of *Muræna helena* given by Schmidt and apart from the similarity in these characters, the close resemblance between Larva I and the larva of *M. helena* in general appearance also justifies the assumption that the egg and larvæ described by Nair and Bhimachar belong to Muræ-nidæ. As in the case of the ophichthyid egg and larva, it is not possible to say whether the murænid egg and larva belong to the same species since the



PHOTOS. 1-2

embryo is not fully formed and does not show the full complement of myotomes which is generally seen in the final stage of embryonic development only.

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Explanation of Plate

Photograph 1. One-day old larva.

Photograph 2. Three-day old larva.

GENERAL REMARKS ON INDIAN LEPTOCEPHALI*

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Introduction

The seas around India are fairly rich in varieties of eels and, therefore, it is not surprising that several kinds of leptocephali have been recorded in recent years from the coastal waters. Confining our attention to the leptocephali collected from the Indian coastal waters only, it may be said that Kaup's account (1856) of *Leptocephalus acuticaudatus* and *Leptocephalus dussumieri* collected from Malabar, *Leptocephalus tænia* from India and Maldives and *Leptocephalus marginatus* from Pondicherry may be considered to be the pioneer attempt in this field. Kaup's account of these larvæ are very brief and the figures given by him serve to give an idea of the general appearance. Bertin (1935) re-examined the types of leptocephali described by Kaup which were kept in the Paris Museum and gave additional information about the different species. But Bertin's account shows that there is a mix-up of Kaup's type leptocephali in the Paris Museum and consequently it is difficult to decide whether his redescription of the species is applicable to the leptocephali collected from India. To cite instances, Kaup's description of *L. acuticaudatus* is based on a single specimen sent by Dussumier from Malabar while Bertin states that 2 specimens were sent by Dussumier from the Coromandel Coast. Similarly Bertin in his account states that Kaup has recorded 4 specimens of *L. dussumieri* in the Paris Museum whereas Kaup records only 3 examples of this species. Bertin further mentions that the single specimen of *L. dussumieri* sent by Dussumier from Malabar cannot be found in the collection. In regard to *L. tænia*, Kaup's material consisted of 8 examples of which 4 were sent by Dussumier—2 from India and 2 from Maldives. According to Bertin the collection contains only one specimen sent by Dussumier from Maldives which has become broken up into fragments making it impossible to measure it or count the myotomes. A single

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specimen of *L. marginatus* was sent to the Museum by Leschenault from Pondicherry under the name of Kouleny which according to Bertin is stuck on a glass and preserved dry so that it is rendered quite useless. It is seen from the above account that the Indian specimens of the 4 species of leptocephali which were collected more than a century back and described by Kaup were either misplaced or rendered useless with the exception of *L. acuticaudatus*. The information given by Kaup and Bertin about the place of collection of this leptocephalus also does not tally and, therefore, it is difficult to make out the exact origin of the specimen. However, based on their accounts, it is possible to refer *L. acuticaudatus* to its parent form, namely, *Uroconger lepturus* (Nair, 1946). Regarding *L. marginatus* and *L. tænia*, it may be stated that since the re-examination of the material has definitely shown that the Indian examples are no longer useful and Bertin's redescription of the two species is entirely based on material received from other places in the Atlantic and Pacific Oceans, his account cannot be considered as applicable to the Indian specimens. It is, therefore, found necessary to make use of Kaup's account and figures of these two species even though they lack many of the essential characters useful for identification. Ancona (1928) pointed out that *L. tænia* collected from the Indian Ocean is referable to *Ophisoma anago* (*Congrellus anago*) and it is highly probable that *L. marginatus* also belongs to the same species. There is close agreement in size, nature of the fins and colouration between these larvæ and the leptocephalus of *Congrellus anago* described by Gopinath (1946 and 1950) and Nair (1948). *L. marginatus* is obviously the edentulous stage in the metamorphosis where the vertical fins are better developed than in *L. tænia* which is believed by Kaup to show the vertical fins in the fresh state, even though the specimens in the Museum do not show any of the fins. Kaup records the absence of the pectoral fin in both the species. In the leptocephalus of *C. anago* the "pectorals are feebly developed" (Gopinath, 1950) and it is likely that in *L. tænia* the pectoral fins have shrivelled up due to imperfect preservation as in the case of its other fins. Kaup further states that in *L. marginatus* the fin rays have been noticed in the tail region only and this condition also shows a very close resemblance to the leptocephalus of *C. anago* where the "dorsal fin is very short with closely set developing fin rays, while the anal is slightly longer with rays well developed and prominent" (Nair, 1948). Regarding the pigmentation of *L. tænia* and *L. marginatus* Kaup mentions the presence of pigment cells on the dorsal and anal fins and in transverse rows under the lateral line. Gopinath (1950) records a similar pattern of pigmentation for the two vertical fins of the larva of *C. anago* and further states that "each intersegmental septum, along its lower half has a row of

small chromatophores arranged on it " which shows the almost perfect similarity in the mid-lateral pigmentation of *L. tænia*, *L. marginatus* and the leptocephalus of *C. anago*. It may be pointed out that the mid-lateral pigmentation composed of transverse rows of chromatophores is a characteristic feature of the larva of *C. anago*. This close agreement in the pigmentation taken in conjunction with the size and nature of the fins shows that the Indian specimens of *L. tænia* and *L. marginatus* studied by Kaup could belong to *C. anago* only. Ancona (1928) suggested that *L. dussumieri* belongs to the family Murænidæ owing to the absence of pectoral fins and this identification appears to be correct based on the other characters as well. Kaup described the larva as a " moderately elongated species, whose transverse muscular furrows are very fine, and not near each other " " with no dots either on the lateral line or along the edge of the belly " and the figure given by him tallies well with the general shape of the murænid leptocephali. The absence of teeth in *L. dussumieri* indicates that it is in the early metamorphosing stage.

Day (1865) collected *Leptocephalus malabaricus* from Cochin and the brief description given by him is not adequate for its identification.

Southwell and Prashad (1919) described two new species of larvæ, namely, *Leptocephalus milnei* and *Leptocephalus vermicularis* collected in a small beam trawl at Doorakara, Sunderbans (Gangetic Delta). Owing to the similarity in the myotome number, *L. vermicularis* was considered by the subsequent workers as an advanced elver stage of *L. milnei* (Ancona, 1928; Nair, 1947; Gopinath, 1950; Jones and Pantulu, 1952). During the course of this comparative study, an important character relating to the length of the dorsal fin which was not mentioned by Southwell and Prashad, was detected from the illustrations. From the figures of the larvæ, it is seen that the dorsal fin in *L. milnei* is very long and occupies nearly the posterior $\frac{3}{4}$ of the larva while in *L. vermicularis* it is very short and occupies only the posterior $\frac{1}{4}$ of the larva. It is known that the length of the dorsal fin increases during metamorphosis and because of the shortness of this fin in *L. vermicularis*, it cannot be an elver stage of *L. milnei*, but belongs to a distinct species. Based on the study of the characters and body proportions it is now possible to say that *L. vermicularis* and *L. milnei* belong to the families Ophichthyidæ and Murænidæ respectively. The ophichthyid character of the former larva, which is nearing the elver stage, is seen from the shortness of the dorsal fin and in the characteristic arrangement of the chromatophores which are " specially collected in groups on the ventral surface of the body". The general shape, greater height and other body measurements taken in conjunction with the pigmentation which is " confined to the base of the vertical fins" and the absence of mid-lateral row of chromatophores show that

L. milnei belongs to the family Murænidæ. The larva, however, possesses pectoral fins which are very small and rounded and such rudimentary pectoral fins have been noted in other murænid larvæ as well (Nair, 1947 and 1948; Jones and Pantulu, 1952).

The Ceylon leptocephali described by Deraniyagala (1934) consist of 9 varieties collected from the pearl banks in the Gulf of Mannar and Wadge Bank off Cape Comorin. Most of the larvæ were taken at night time with a dip net from the surface layers with the exception of Larva V which is a preleptocephaline stage obtained from an all-night townet collection made at the surface. He pointed out some of the important characters of the larvæ and tentatively assigned Larva I to Congridæ, Larva IV to *Muræsox*, Larva VI to Ophichthyidæ, Larva VII to Moringuidæ and Larvæ VIII and IX to Muræsidæ. Deraniyagala assigned Larva IV to the genus *Muræsox* on account of the rostral constriction and tubulate nature of the anterior nares. This identification was later confirmed by Jones and Pantulu (1952). The descriptive account of the larva given by Deraniyagala is too brief for comparison. However, the figures given by him show interesting features which are not generally found in any other Indian leptocephali and much less in those of *Muræsox*. Deraniyagala mentions the presence of 3 short mandibular teeth in the larva, which obviously represent a few of the larval teeth, the others perhaps being shed prior to metamorphosis. The nature of the teeth and the important morphometric characters of the larva which have been calculated from the figures indicate that the larva corresponds to Stage II of *Muræsox cinereus* (Nair, 1947). An interesting feature of Larva IV is the highly tubular nature of the anterior nostril which is seen clearly in the edentulous stage itself. None of the larval eels recorded so far from the Indian waters show this condition and in *Muræsox* it becomes tubular in the elver stage only (Nair, 1947; Nair and Mohamed, 1960 *a* and 1960 *b*). It is also seen that the predorsal distance in Larva IV is higher than the preanal distance and these measurements are 50·4% and 49·6% respectively. In the corresponding stage of the 3 larvæ of *Muræsox*, the predorsal distance is very short and it is only $\frac{1}{3}$ to $\frac{1}{4}$ of the preanal distance. The most significant difference is seen in the pigmentation of the Ceylon larva. The figure of the larva shows clearly that the mid-lateral chromatophores commence from the head region and the pigment cells are widely distributed at intervals of several myotomes. The figure also shows the presence of two more rows of pigment cells; one situated above the mid-lateral row and extending throughout the length of the larva while the other, found below the mid-lateral row, is confined to the posterior region of the larva. The presence of more than one row of body chromatophores has not so far been recorded in any other larvæ

occurring in Indian waters. These distinctive characters of Larva IV described by Deraniyagala, especially the presence of tubular anterior nostril in the leptocephalus stage itself and 3 longitudinal rows of pigment cells on the body, show that the larva does not belong to *Muræsesox* but to some other group of eels. Larva V which was collected off Cape Comorin and described by Deraniyagala, is a preleptocephaline larval stage measuring 20 mm. in length. Even though the looped nature of the alimentary canal is not described by him, the figure shows clearly 9 loops in the alimentary canal and, therefore, it is assigned to the family Ophichthyidæ (Nair and Dharmamba, 1960).

All the leptocephali described by Nair from Madras (1946 and 1947) were obtained from the Madras plankton and in the case of *Muræsesox cinereus* and *Murtena macrura* the correct identification was made possible by their metamorphosis in the Laboratory. *Uroconger lepturus* was identified by using the usual method of myotome and vertebral counts supplemented by the other important characters found common to the larva and the adult.

The leptocephalus of *Congrellus anago*, *U. lepturus*, *M. cinereus* and *Muræna* sp. were recorded from the Gulf of Mannar where they were observed to be present in good numbers in the shore seine catches which were operated at Kutikal Point, Rameswaram Island (Nair, 1948).

Nair and Bhimachar (1950) collected two preleptocephaline larvæ from Tholayiram Paar, one of the pearl banks situated off Tuticorin in the Gulf of Mannar. These preleptocephaline larvæ which represent very early stages, belong to the families Murænidæ and Ophichthyidæ (Nair and Dharmamba, 1960).

Gopinath's account (1950) of the leptocephali of the Trivandrum coast deals with 6 larvæ collected mostly from the fishermen's catches. Leptocephalus A and Leptocephalus F alone were obtained from the townet collections while the rest were obtained from the shore seines operated in the coastal waters. Out of the 6 larvæ described by him, 2 belong to *Congrellus anago* (Gopinath, 1946; Nair, 1948) and *Muræsesox cinereus* (Nair, 1947). He referred Leptocephalus A and Leptocephalus C to the family Ophichthyidæ due to the presence of a free tail. Leptocephalus F described by him is also an ophichthyid as shown by the shortness of the dorsal fin, free nature of the tail and the characteristic pigmentation in the form of black patches. Leptocephalus E described by Gopinath shows characteristic murænid features in body proportions and in the absence of the mid-lateral

chromatophores about which he states that "the large elongate chromatophores found along the myocomma in most of the other species are wanting in this". The rudimentary nature of the pectoral fin is also recorded by him.

Jones and Pantulu (1952) collected the metamorphosing stages of *Muræsox talabon* from the Burhabulong estuary in Orissa State along with 3 other murænid leptocephali and doubtfully assigned another murænid leptocephalus collected from the Hooghly estuary to *Muræna tile*. The larval stages assigned by them to *M. talabon* have been shown by Nair and Mohamed (1960 *b*) to belong to *Muræsox cinereus* already described by Nair (1947). These authors (1954) provisionally confirmed the identification of the leptocephalus of *Muræna tile* and described its metamorphosing stages along with 2 growing stages of two other murænid leptocephali. The different murænid leptocephali described by these authors have been discussed in detail by Nair and Mohamed (1960 *e*). Jones and Pantulu (1955) described 5 varieties of ophichthyid leptocephali of which one is in the early preleptocephaline stage. Of the remaining 4 larvæ, one was collected from Madras, 2 from Travancore and the last one which they referred to *Pisoodonophis hijala* from the Orissa coast. The two Travancore larvæ were collected from the shore seine catches while that of *P. hijala* was collected in a sub-surface townet haul made in the coastal waters. The ophichthyid leptocephali from Madras are also believed to be obtained from townet collections.

Classification

Altogether 47 different varieties of leptocephali have been recorded so far from the Indian coastal waters of which only those described by Kaup (1856), Day (1865) and Southwell and Prashad (1919) were given scientific names while the others were either correlated with the adults or designated by the different authors with letters and numbers, even though they could be given new scientific names. Out of these 47 varieties of leptocephali 7 are considered synonymous with other varieties as shown below:—

1. *Leptocephalus acuticaudatus* Kaup, 1856	Leptocephalus of *Uroconger lepturus*. Nair, 1946 and 1948; Nair and Mohamed, 1960 *c*.
2. *Leptocephalus tænia* Kaup, 1856	Leptocephalus of *Congrellus anago*. Gopinath, 1946 and 1950; Nair, 1948.
3. *Leptocephalus marginatus* Kaup, 1856	Do.

4. Leptocephalus of *Muræsox talabon*. Jones and Pantulu, 1952	Leptocephalus of *Muræsox cinereus*. Nair, 1947 and 1948; Gopinath, 1950.
5. Leptocephalus III. Jones and Pantulu, 1952	Leptocephalus II. Jones and Pantulu, 1952.
6. Muræna II. Pantulu and Jones, 1954	Leptocephalus of *Muræna tile*. Jones and Pantulu, 1952; Pantulu and Jones, 1954.
7. Muræna III. Pantulu and Jones, 1954	Do.

It is interesting to note that out of the 40 valid species, the following 8 leptocephali have been referred to their respective adults and the metamorphosis of 6 species has been studied in detail:—

1. *Uroconger lepturus* .. Nair, 1946; Nair and Mohamed, 1960 *c*
2. *Muræsox cinereus* .. Nair, 1947
3. *Muræna macrura* .. Nair, 1947
4. *Congrellus anago* .. Gopinath, 1946 and 1950; Nair, 1948
5. *Muræna tile* .. Jones and Pantulu, 1952; Pantulu and Jones, 1954
6. *Pisoodonophis hijala* .. Jones and Pantulu, 1955
7. *Muræsox talabonoides* .. Nair and Mohamed, 1960 *a*
8. *Muræsox talabon* .. Nair and Mohamed, 1960 *b*

All the remaining 32 larvæ have been referred to their respective families with the exception of 5 larvæ, namely, *Leptocephalus malabaricus* (Day, 1865), Larva II, Larva III and Larva IV (Deraniyagala, 1934) and Leptocephalus V (Nair and Mohamed, 1960 *e*). The family-wise grouping of the different unidentified leptocephali are given below:—

Family: OPHICHTHYIDÆ

1. *Leptocephalus vermicularis*, Southwell and Prashad, 1919.
2. Larva V, Deraniyagala, 1934.
3. Larva VI, Deraniyagala, 1934.
4. Larva II, Nair and Bhimachar, 1950.
5. Leptocephalus A, Gopinath, 1950.
6. Leptocephalus C, Gopinath, 1950.

7. Leptocephalus F, Gopinath, 1950.
8. Prolarva (Madras), Jones and Pantulu, 1955.
9. Leptocephalus (Madras), Jones and Pantulu, 1955.
10. Leptocephalus I (Travancore), Jones and Pantulu, 1955.
11. Leptocephalus II (Travancore), Jones and Pantulu, 1955.
12. Leptocephalus I, Nair and Mohamed, 1960 *d*.
13. Leptocephalus II, Nair and Mohamed, 1960 *d*.
14. Leptocephalus III, Nair and Mohamed, 1960 *d*.
15. Preleptocephaline Larva, Nair and Dharmamba, 1960.

Family: MURÆNIDÆ

16. *Leptocephalus dussumieri*, Kaup, 1856.
17. *Leptocephalus milnei*, Southwell and Prashad, 1919.
18. Larva VIII, Deraniyagala, 1934.
19. Larva IX, Deraniyagala, 1934.
20. *Muræna* sp. Nair, 1948.
21. Larva II, Nair and Bhimachar, 1950.
22. Leptocephalus E, Gopinath, 1950.
23. Leptocephalus I, Jones and Pantulu, 1952.
24. Leptocephalus II, Jones and Pantulu, 1952.
25. Leptocephalus IV, Nair and Mohamed, 1960 *e*.

Family: CONGRIDÆ

26. Larva I, Deraniyagala, 1934.

Family: MORINGUIDÆ

27. Larva VII, Deraniyagala, 1934.

Others

28. *Leptocephalus malabaricus*, Day, 1865.
29. Larva II, Deraniyagala, 1934.
30. Larva III, Deraniyagala, 1934.
31. Larva IV, Deraniyagala, 1934.
32. Leptocephalus V, Nair and Mohamed, 1960 *e*.

DISTRIBUTION

It is seen that the ophichthyid leptocephali are more common in the coastal waters and 16 larvæ have been recorded so far, including the 4 pre-leptocephaline stages described by Deraniyagala (1934), Nair and Bhimachar (1950), Jones and Pantulu (1955) and Nair and Dharmamba (1960) and the leptocephalus of *Pisoodonophis hijala* (Jones and Pantulu, 1955). Murænid larvæ are also common with 12 valid species recorded till now from the coastal waters and estuaries including the preleptocephaline stage recorded by Nair and Bhimachar (1950) and the leptocephali of *Muræna macrura* (Nair, 1947) and *Muræna tile* (Jones and Pantulu, 1952; Pantulu and Jones, 1954). Among the other leptocephali 3 belong to *Muræsox*, one each to *Uroconger*, *Congrellus*, Congridæ and Moringuidæ. Ophichthyid larvæ appear to be more common along the West coast as shown by the higher number of larvæ recorded from this coast. Out of the 12 known leptocephali, 8 are from the West coast of which 5 are from the Travancore coast and 3 from Bombay coast. The 4 ophichthyid larvæ recorded from the East coast are from the Gulf of Mannar and Madras, Orissa and Bengal coasts. On the other hand, the record of murænid leptocephali shows that they are more common on the East coast and out of the 11 valid species, only 3 are from the West coast collected from Trivandrum, Malabar and Bombay coasts while the 8 larvæ recorded from the East coast are from the Gulf of Mannar and Madras, Orissa and Bengal coasts. It is interesting to point out that, barring the 4 murænid leptocephali collected from the estuaries of Orissa and Bengal, all the others were from the coastal waters only. It is difficult to say with our present knowledge, whether the murænid leptocephali collected from these estuaries show an actual migration or whether they are brought into the estuaries from the coastal waters by the action of the tide. Some of the larvæ have been recorded from both the coasts and the leptocephalus of *Uroconger lepturus* appears to be a widely distributed species and it has been recorded in good numbers from Madras, Gulf of Mannar, Bombay and Malabar (?). *Muræsox cinereus* is also widely distributed and has been recorded from Orissa, Madras, Gulf of Mannar and Trivandrum. *Congrellus anago* appears to have a restricted distribution and it is recorded so far only from Pondicherry, Gulf of Mannar and Trivandrum and Maldives while the other recorded leptocephali are known only from the place of collection of the type specimens.

It is interesting to point out here that most of the leptocephali recorded so far, were collected from the shallow waters either from the fishermen's catches or inshore plankton collections during the months January to May

which perhaps constitute the period when the fully-grown larvæ enter the shallow coastal waters in large numbers and metamorphose into the elvers. This is clearly demonstrated by the paucity of toothed larvæ and the abundance of edentulous stages of species like *Congrellus anago*, *Uroconger lepturus*, etc. The abundance of metamorphosing leptocephali during this period also indicates the possibility that the Indian eels have a definite spawning period. There is no record of the eggs, preleptocephaline and early larval stages of any of the identified larvæ and there is no information about the time taken by the leptocephalus to reach the maximum size. Aiyer, Unny and Varkey (1944) noted the occurrence of eel eggs in the Madras plankton, but the details of the collection are not available. Similarly Bapat (1955) also recorded the presence of eel eggs in the plankton collections made from the Gulf of Mannar and Palk Bay without giving the dates of occurrence. However, the eggs and larvæ recorded from the Gulf of Mannar by Nair and Bhimachar (1950) and the Lawson's Bay by Nair and Dharmamba (1960) were collected in April and February respectively. The preleptocephaline stages described by Deraniyagala (1934) and Jones and Pantulu (1955) were collected in November and December respectively. Mature, oozing and spent individuals of *Muræsox talabonoides* were observed from January to April in the trawler catches made off Bombay. These stray records of the eggs and larvæ do not give any indication about the breeding season; but taken in conjunction with the gonadial condition noted in *M. talabonoides* suggest the possibility that the breeding season of Indian marine eels is likely to be from November to April. It is very unlikely that the leptocephali will reach the full growth within the spawning period itself, but on the other hand it is possible that the duration of the leptocephalus stage is about one year or more.

Morphometric and Other Characters

The Indian leptocephali show great diversity in size and the longest larva recorded so far is that of *Congrellus anago* which attains a maximum size of 158 mm. (Gopinath, 1950). The leptocephalus of *Uroconger lepturus* and a few ophichthyid larvæ like Leptocephalus I from Bombay (Nair and Mohamed, 1960 *d*) are also relatively long, but the smallest size recorded so far is seen in Larva VII (Deraniyagala, 1934) which measures only 42·5 mm. As a general rule the murænid leptocephali are very tall while those of *Muræsox* and *Congrellus* show moderate height only. The larva of *Uroconger lepturus* and those of the family Ophichthyidæ are relatively very short. The maximum height recorded so far is in Leptocephalus E of Gopinath (1950) which measures 19·1% or nearly 1/5th of the total length.

On the other hand, the minimum height of 4·1% is noticed in the ophichthyid Leptocephalus I collected from Bombay waters (Nair and Mohamed, 1960 *d*).

A similar comparison of the length of the head of the different leptocephali is rendered difficult owing to the fact that there are quite a few larvæ which show signs of metamorphosis and consequently possess higher values for the head due to its elongation. However, it is seen that the head of *Congrellus anago* is the smallest and measures only 2·8%. Generally the head of ophichthyid leptocephali is also short measuring only 3·4% as seen in the Leptocephalus F of Gopinath (1950) and Leptocephalus I (Nair and Mohamed, 1960 *d*). The head of the larva of *Uroconger lepturus* is of moderate length while those of *Muræsox* spp. and Muræsidæ are usually long and the longest head is seen in the moringuid Larva VII of Deraniyagala (1934) which measures 9·5% (calculated from the description).

The length of the snout is very long in the genus *Muræsox* where the maximum size of 44·0% is seen in the larva of *M. cinereus*. The snout is generally small in the family Muræsidæ and the smallest snout is seen in Leptocephalus I (Jones and Pantulu, 1952) and it measures only 18·0% of the head. This character varies from ¼ to ⅓ the length of head in the larvæ belonging to the other families.

The diameter of the eye shows slight variation in the different leptocephali. In the leptocephali of the family Muræsidæ and those of *Congrellus anago* and *Uroconger lepturus*, the eye is about ¼ the length of the head, while in the family Ophichthyidæ and *Muræsox* it is about 1/5th only. In actual size the eye of Leptocephalus V (Nair and Mohamed, 1960 *e*) shows the maximum diameter of 1·4 mm. while the smallest diameter of 0·5 mm. is seen in the muræsnid Leptocephalus I (Jones and Pantulu, 1952), ophichthyid leptocephalus from Madras and the larva of *Pisoodonopthis hijala* from Orissa (Jones and Pantulu, 1955).

In regard to the number of teeth, the larva of *Uroconger lepturus* is peculiar in showing the highest number of 23/15 teeth. Very low number of teeth has been recorded in a few leptocephali, but owing to the very brief description of the larvæ given by the authors, it is difficult to say whether they belong to the larval set or whether they represent the rudiments of the adult set of teeth which are in the course of formation.

Individual variation in the myotome number is found in almost all the species and the range of variation is as low as one myotome in the case of Leptocephalus II (Nair and Mohamed, 1960 *d*) while it is very high in *Uroconger lepturus* and *Muræsox talabon* with 19 and 10 myotomes respectively.

The range given for *U. lepturus* does not represent the normal condition since regeneration of the tail takes place in this species. The myotome number of species belonging to the same family overlaps only in the case of Leptocephali I and II (Nair and Mohamed, 1960 *c*) and in the larvæ of *Muræne-sox talabonoides* (Nair and Mohamed, 1960 *a*) and *Muræsox talabon* (Nair and Mohamed, 1960 *b*) and this is caused by the higher myotome variation found in Leptocephalus I and *M. talabon*. The maximum number of 223 myotomes is seen in the ophichthyid leptocephalus collected from Madras (Jones and Pantulu, 1955). Another ophichthyid larva, Leptocephalus C of Gopinath (1950) also shows a high number of 206 myotomes while in all the other leptocephali it is very much less excepting some murænid leptocephali and *Leptocephalus malabaricus* (Day, 1865). The minimum number of myotomes recorded for any Indian leptocephali is seen in Larva IX of Deraniyagala (1934) which shows only 101 myotomes. According to the myotome number, the different larvæ of the family Muræniдæ come under two groups. Leptocephalus of *Muræna macrura* (Nair, 1947), *Muræna* sp. (Nair, 1948), Leptocephalus I and Leptocephalus II (Jones and Pantulu, 1952) show high myotome number varying from 205–216. This group characterised by high myotome number may be appropriately termed as the ' macrura group ' after the species whose adult is known. The other group comprising of the leptocephalus of *Muræna tile* (Jones and Pantulu, 1952; Pantulu and Jones, 1954), *Leptocephalus milnei* (Southwell and Prashad, 1919), Larva VIII (Deraniyagala, 1934) and Leptocephalus E (Gopinath, 1950) possess low myotome number ranging from 120–126 and may be called the ' tile group ' after *M. tile*. Generally the ' macrura group ' shows more than 200 myotomes while the ' tile group ' shows less than 130 myotomes, the exceptions being *Leptocephalus dussumieri* (Kaup, 1856) and Leptocephalus IV (Nair and Mohamed, 1960 *e*) which exhibit an intermediate condition and Larva IX (Deraniyagala, 1934) which shows the lowest myotome number recorded so far. The characteristic pigmentation round the pupil of the eye of the ' macrura group ' and their absence in the ' tile group ' strengthens this grouping of the murænid larvæ. As a general rule the myotomes of murænid leptocephali are very narrow and compactly arranged while the broadest myotomes measuring as much as 1·5 mm. in width are seen in the leptocephalus of *Congrellus anago* (Nair, 1948).

The predorsal distance also shows marked difference among the different leptocephali indicating that this character serves as a distinguishing feature of the leptocephali of at least a few families. The ophichthyid leptocephali invariably show the highest predorsal distance seen among the different leptocephali and this is clearly seen from the high value of 95·7% observed in

Leptocephalus III (Nair and Mohamed, 1960 *d*). Table II given by Nair and Mohamed (1960 *d*) shows clearly that in almost all the ophichthyid leptocephali the predorsal distance is above 90% of the length. The predorsal distance of the ophichthyid Leptocephalus A, Leptocephalus C and Leptocephalus F of Gopinath (1950) is not available; however, the dorsal fin is described as very short in these larvæ indicating that the predorsal distance is long as in the case of the other ophichthyid larvæ. This distance in the larva of *Congrellus anago* also is very long and measures 84·5% of the length (Nair, 1948). The predorsal distance in murænid leptocephali shows variation and it is nearly half the length of the larva in most cases. In the genus *Muræsox*, this distance is only about $\frac{1}{4}$ of the length with *M. talabonoides* showing the shortest distance of 17·0% recorded so far. The predorsal distance of Larva III of Deraniyagala (1934) is also very short and measures only 17·3% (calculated from the description).

The preanal distance which serves to indicate the relative length of the alimentary canal, is found to vary among the different larvæ. The longest preanal distance is seen in Larva I of Deraniyagala (1934) where it forms 92·6% of the length (calculated from the text-figure). The larva of *Muræna tile* (Muræna II of Pantulu and Jones, 1954) also shows a high value of 83·8%. In most of the other murænid larvæ this distance occupies more than $\frac{3}{4}$ the length of the larvæ. The preanal distance in the larva of *Uroconger lepturus* is also long and forms 73·2% of the length. This distance is about $\frac{2}{3}$ the length in *Congrellus anago* and *Muræsox* spp. while in the ophichthyid leptocephali it is generally short and extends only up to the middle of the larva. The shortest preanal distance is found in the ophichthyid leptocephalus from Madras (Jones and Pantulu, 1955) where it is only 38·1% of the length.

The highest number of preanal myotomes of 126 is seen in *Muræna* sp. (Nair, 1948) while the lowest number of 51 preanal myotomes is seen in Leptocephalus V (Nair and Mohamed, 1960 *e*). This, no doubt, is a variable character depending on the stage of growth as well as metamorphosis of the leptocephalus. The larvæ of Murænidæ comprising of both the 'macrura group' and 'tile group' generally show a high preanal myotome number in contrast with the relatively lower number found in the other families.

The alimentary canal of all the larvæ excepting those of the family Ophichthyidæ is of uniform thickness and takes a straight course. The characteristic features of the alimentary canal shown by the ophichthyid leptocephali are of diagnostic importance. The alimentary canal of most of the ophichthyid larvæ is straight and shows hump-like thickenings

at regular intervals except in Larva IV of Deraniyagala (1934), Leptocephalus A of Gopinath (1950) and Leptocephalus III (Nair and Mohamed, 1960 *d*) in which the alimentary canal shows a looped condition. Similar hump-like thickenings of the alimentary canal have also been observed in these 3 larvæ with looped alimentary canal and they are found at the angles of the loops. It is quite likely that the leptocephali with looped alimentary canal may belong to a distinct group from those possessing a straight alimentary canal with hump-like thickenings.

The paired pectoral fins and the median vertical fins show difference in shape and size which are useful in the identification of the different families as well as the species. The pectoral fin is of taxonomic importance in the identification of the leptocephali and elvers of the 3 species of *Muræsox*. In the larva of *M. cinereus*, the pectoral fin is perfectly circular and it becomes oval in the elver stage. The pectoral fin of *M. talabon* is oval in the leptocephalus and becomes long and pointed in the elver stage. This fin is long and pointed in the larval and elver stages of *M. talabonoides*. The presence of rudimentary pectoral fin has been shown in the larvæ of *Muræna macrura* (Nair, 1947), *Muræna tile* (Jones and Pantulu, 1952; Pantulu and Jones, 1954), etc., and it is likely that the other murænid leptocephali may also show the fin in the early larval stages. It may be mentioned that Schmidt (1913) pointed out that " all murænoids have larval pectoral fins, even those in which they disappear later; the time for their disappearance being very variable in different species". The pectoral fin of the leptocephalus of *Congrellus anago* is feebly developed with indistinct rays.

The dorsal fin is found to be extremely short in almost all the ophichthyid leptocephali and in some larvæ the posteriormost rays excepting the last few ones are slightly longer than the rest. The corresponding rays of the anal fins are also longer and consequently these regions of the two fins are slightly wider than the other regions of the fins. In Leptocephalus III (Nair and Mohamed, 1960 *d*) and Leptocephalus V (Nair and Mohamed, 1960 *e*) these rays are considerably longer than the others and show a uniform zig-zag curvature at their tips.

The anal fin in almost all the leptocephali commences just behind the anal opening and the only exception appears to be Larva VIII (Deraniyagala, 1934) belonging to the family Moringuidæ, where both the dorsal and anal fins are confined to the tail region.

The caudal fin is well developed in all the leptocephali of the different families except in the family Ophichthyidæ which is characterised by the absence of this fin.

PIGMENTATION

The pigmentation of the leptocephali of the different families have been discussed in detail under the respective sections (Nair and Mohamed, 1960 *b*, 1960 *d*, 1960 *e*); however, the important features useful in the identification of the families may be mentioned here. The normal head pigmentation consisting of a few chromatophores on the upper jaw and also in the heart region, is present in most of the leptocephali of the different families except in *Congrellus anago*, the head of which is devoid of pigmentation. Head pigmentation of diagnostic importance is rare in Indian leptocephali and so far it is observed only in *Leptocephalus malabaricus* (Day, 1865), leptocephalus of *Uroconger lepturus*, ' macrura group ' of murænid leptocephali and in Leptocephalus IV (Nair and Mohamed, 1960 *e*). The head of *L. malabaricus* is " golden; lower surface orange. One line of yellow, spotted with green proceeds backwards from the eye; a second from the mouth; this last one also traverses the lower lip". The group of black chromatophores found below the eye is a characteristic feature of the leptocephalus of *U. lepturus* and this character together with the pointed and whip-like tail enables its easy identification. The 8-stellate chromatophores found around the pupil of the eye is recorded only in the ' macrura group ' and may prove to be a feature of this group of murænid leptocephali. The only exception is Leptocephalus I, about which Jones and Pantulu (1952) state that " as this specimen was examined only after a few months after it was collected it is difficult to say whether any chromatophores around the pupil identical to those occurring in specimens II and III were present". The groups of black pigment cells found on either side of the eye of Leptocephalus IV (Nair and Mohamed, 1960 *e*) give the head a prominent dark colouration and it is possible that it has specific significance.

The mid-lateral pigmentation is not generally found in the murænid leptocephali and also in Leptocephalus V (Nair and Mohamed, 1960 *e*). This pigmentation is very conspicuous in the family Ophichthyidæ even though variation in the number and arrangement of the pigment cells is found among the different species. The presence of post-anal and mid-lateral groups of chromatophores in Leptocephalus A of Gopinath (1950) and in Leptocephalus I and Leptocephalus II (Nair and Mohamed, 1960 *d*) are additional features found only in these larvæ. The mid-lateral pigmentation is moderate in the genera *Muræsox* and *Uroconger* while in *Congrellus* it is in the form of transverse rows of chromatophores arranged along the lower half of the myocommas. Larva IV of Deraniyagala (1934) is peculiar in showing 2 more additional rows of chromatophores situated on either

side of the mid-lateral row of pigment cells. In Larva VII of Deraniyagala (1934) the mid-lateral pigmentation is absent, but in its place a few large stellate melanophores, whose number is variable, is seen along the lateral line.

The characteristic features in the pigmentation of the alimentary canal are of considerable importance in the identification of the families and even species. The difference in the pattern of pigmentation found within the same genus is clearly seen in the 3 larvæ of *Muræsox* (Nair, 1947; Nair and Mohamed, 1960 *a*, 1960 *b*) and to some extent in the larvæ of the family Ophichthyidæ, where the number of pigment groups on the hump-like thickenings of the alimentary canal and also those along the post-anal, mid-lateral line appears to indicate species variation only.

The anal fin in all the leptocephali is pigmented while the dorsal and caudal fins show variations in the pigmentation in the leptocephali belonging to the different families and sometimes within the same family itself.

Growth

There is no record relating to growth in leptocephalus except the 2 stages described by Pantulu and Jones (1954) for *Muræna tile* (Stages I and II of Muræna II and III). As in the case of *Uroconger lepturus*, a study of the changes undergone during the growth of this murænid larva is not possible with the single intermediate stage described by them. However, certain generalisations are possible as a result of the study of the different stages available in the case of *Muræsox talabonoides* (Nair and Mohamed, 1960 *a*) and Leptocephalus I (Nair and Mohamed, 1960 *d*). Among the morphometric characters, the increase in the length of the larva is rapid while the other body measurements do not generally show proportionate increase in the growing stages. On account of this, the height and length of head show decreasing percentages, in spite of their growth during this period. A similar decrease in the height is also seen in the metamorphosing stages consequent on the reduction in the height while the length of head increases considerably during the transformation. The marked change in their values in the different metamorphosing stages is also partly caused by the reduction in the length. The predorsal and preanal distances in the leptocephalus of *Muræsox talabonoides* show gradual reduction in the growing as well as metamorphosing stages. But in Leptocephalus I (Nair and Mohamed, 1960 *d*) no reduction of the predorsal and preanal distances is noticed in the growing phase since these distances also register proportionate increase and consequently their percentages are fairly constant during growth. It is likely that this feature is

found only in the family Ophichthyidæ since reduction in these distances is also seen in the leptocephali of *Uroconger lepturus* (Nair and Mohamed, 1960 *c*) and *Muræna tile* (Stages I and II of Muræna II and III, Pantulu and Jones, 1954). Contrasting conditions are found in regard to the length of the snout during the growth of the leptocephalus of *Muræsox talabonoides* (Nair and Mohamed, 1960 *a*) and Leptocephalus I (Nair and Mohamed, 1960 *d*). The former shows higher percentages during the growing phase, while its values decreases in the successive metamorphosing stages. The higher value for the snout no doubt is caused by its elongation which is not proportionate to that of the head. The length of the snout of Leptocephalus I (Nair and Mohamed, 1960 *d*) also records increase during the growing phase, but at a lower rate than that of the head and hence shows decreasing percentages. There is no change in the size of the eye, but its diameter shows decreasing percentage values in both the species during the growing phase and the values continue to decrease in *M. talabonoides* during the metamorphosing phase also, since the head elongates in both these phases.

It is interesting to note that the preanal number of myotomes is more or less constant in the growing stages of Leptocephalus I (Nair and Mohamed, 1960 *d*) while reduction is seen in *M. talabonoides* indicating that the anus migrates during the growing phase itself. This phenomenon is also observed in the leptocephali of *Uroconger lepturus* (Nair and Mohamed, 1960 *c*) and *Muræna tile* (Stages I and II of Muræna II and III, Pantulu and Jones, 1954). Normally leptocephali come under two categories depending on the migration of the anus; one showing the migration of the anus during the early larval growing phase while in the other the anus maintains a constant position throughout the larval growing phase. In both these categories the anus migrates anteriorly during the metamorphosing phase only. The leptocephalus of *M. talabonoides*, *U. lepturus* and *M. tile* present a condition in which the migration of the anus takes place even before attaining the full growth of the larva and this has necessarily to be considered as a metamorphosing migration coming under the second category since a shifting of the origin of the dorsal fin also takes place simultaneously. The ophichthyid Leptocephalus I (Nair and Mohamed, 1960 *d*) shows the normal condition found in many leptocephali where the anus maintains a constant position in relation to the myotomes during the growing phase and migrates only during metamorphosis. It is not clear whether the early anal migration noted in these species is caused by the tropical environment which is known to accelerate the metamorphosis of the leptocephali of *Muræsox cinereus* and *Muræna macrura* (Nair, 1947).

METAMORPHOSIS

The leptocephali immediately on reaching full growth begins to metamorphose which is the progressive replacement of the larval characters by the adult ones. The changes undergone in the different leptocephali, whose metamorphosis is known, have been given in detail (Nair, 1947; Nair and Mohamed, 1960 *a*, 1960 *b* and 1960 *c*). A comparative study of the changes in the metamorphosis of the leptocephali belonging to the same genus is possible in the case of *Mutænesox* and *Muræna* only.

No significant variation has been noted in the metamorphosis of the leptocephali of *Muranesox cinereus* (Nair, 1947), *Muranesox talabonoides* (Nair and Mohamed, 1960 *a*) and *Muranesox talabon* (Nair and Mohamed, 1960 *b*) and all of them show the reduction in height and proportionate thickening of the body, the shifting of the vent and the origin of the dorsal fin to an anterior position, the lengthening of the head and the gradual acquisition of the adult colouration. However, it is interesting to point out that these changes occur in the different larvæ in varying degrees indicating perhaps specific differences. Tables II and III (Nair and Mohamed, 1960 *a*) and Table I (Nair and Mohamed, 1960 *b*) show the morphometric characters of the different stages in the metamorphosis of the 3 species and it is seen that the reduction in height during metamorphosis is more or less uniform in all the larvæ with the elver showing the minimum height. The larva of *M. cinereus* records only a minimum reduction by 46·3% of the larval height while *M. talabonoides* and *M. talabon* show greater reduction. The latter species shows the maximum reduction by 64·1% and it may be mentioned here that in the elver, the height of the body is much lower than that of the head. *M. talabonoides* also shows a fairly high reduction of the larval height by 50·6%. It is interesting to note that the two shorter larvæ show more reduction in height than the taller *M. cinereus*. It may be recalled that the predorsal distance is highest in the larva of *M. talabon* while it is lowest in *M. talabonoides*. In the elver stage the maximum predorsal distance is seen in *M. cinereus* while *M. talabonoides* continue to show the minimum distance. It is seen that the maximum reduction of the predorsal distance by 51·4% is seen in *M. talabon* while *M. cinereus* and *M. talabonoides* show reduction by 44·5% and 30·6% respectively. The maximum preanal distance is observed in the elver of *M. talabon* while that of *M. talabonoides* shows the minimum distance. However, the maximum reduction in the preanal distance is seen in *M. talabon* where the original length is reduced by 50·3% while *M. cinereus* and *M. talabonoides* show a reduction by 48·2% and 40·7% respectively.

The head and snout of all the 3 species show fairly uniform increase during the transformation into elver and the greatest snout and head lengths continue to be present in *M. cinereus* while they are shortest in *M. talabonoides*. On the other hand, a complete reversal in regard to the diameter of the eye is seen in the 3 species. It may be mentioned that *M. talabon* possesses the largest larval eye with the smallest eye in *M. cinereus*. In the elver, the eye of *M. cinereus* is the largest while that of *M. talabon*, the smallest among the 3 species. The anterior nostril of *M. talabon* is tubulate though not conspicuous as in the case of *M. cinereus* while in *M. talabonoides*, its tubulate nature is not distinct even in the advanced elvers.

The changes in the shape of the pectoral fin during metamorphosis also show interesting features suggesting that they are useful in the identification of the metamorphosing stages as well. The pectoral fin of the larva of *M. cinereus*, as pointed out earlier, is perfectly circular and it becomes oval in the elver stage. The pectoral fin of *M. talabon* is oval in the larval stage and becomes long and pointed in the elver while that of *M. talabonoides* is pointed and long in both the larval and elver stages. It is seen that the pectoral fins of the leptocephali show a gradation from circular to linear shape in the 3 species.

Even in the assumption of the adult colouration, *M. talabon* occupies an intermediate position only. In *M. cinereus* indications of the adult colouration are seen in the metamorphosing stages while the elver becomes uniformly brownish-black on the dorsal side. In *M. talabon*, the chromatophores on the dorsal side of the elver are somewhat uniformly scattered giving that side a light brown colour while in *M. talabonoides* the pigmentation of the elver is very light and the chromatophores, which are comparatively fewer in number, are widely scattered giving the elver a faint brown colour to the dorsal side.

A similar comparative study of the metamorphosis of the two larvæ of *Muræna macrura* (Nair, 1947) and *Muræna tile* (Pantulu and Jones, 1954) is made easy since only 5 more or less similar stages have been described for both the species. A perusal of Tables I and II which give the characters of the different metamorphosing stages of the two species, shows that considerable reduction in length and height takes place and the reduction in the latter is about the same in both the species.

There is great reduction in the predorsal distance in *M. macrura* by about 78·4% of the larval distance while this reduction is only by 62·3% in *M. tile*. On the other hand, the reduction of the preanal distance is higher in *M. tile*

TABLE I

Measurements of Muræna macrura (*Nair*, 1947)

	Stage I	Stage II	Stage III	Stage IV	Stage V
Total length ..	93·0	77·0	80·0	78·5	69·0
Maximum height	13·0	11·5	8·5	6·5	4·5
(including fins)	(14·0)	(14·9)	(10·6)	(8·3)	(6·5)
Maximum height	12·5	11·0	8·0	6·0	3·0
(excluding fins)	(13·4)	(14·3)	(10·0)	(7·6)	(4·3)
Predorsal distance ..	50·0	40·0	40·0	30·0	8·0
	(53·8)	(51·9)	(50·0)	(38·2)	(11·6)
Preanal distance ..	62·0	53·0	53·0	46·5	32·0
	(66·7)	(68·8)	(66·3)	(59·2)	(46·4)
Length of head ..	4·0	5·0	5·5	6·5	9·0
	(4·3)	(6·5)	(6·9)	(8·3)	(13·0)
Length of snout* ..	1·3	1·6	1·6	1·5	1·3
	(32·5)	(32·0)	(29·1)	(23·1)	(14·4)
Diameter of eye* ..	0·9	1·1	1·0	0·8	0·7
	(22·5)	(22·0)	(18·2)	(12·3)	(7·8)
Total myotomes ..	216	..	..	..	..
Preanal myotomes ..	120	120	120	103	..

* Calculated from the text-figures.

TABLE II

Measurements of Muræna tile (*Pantulu and Jones*, 1954)

	Stage I	Stage II	Stage III	Stage IV	Stage V
Total length ..	62·5	61·0	60·0	55·0	53·0
Maximum height ..	11·6	7·2	6·8	4·5	4·1
	(18·6)	(11·8)	(11·3)	(8·2)	(7·7)
Predorsal distance ..	25·0	21·0	19·0	8·3	8·0
	(40·0)	(34·4)	(31·7)	(15·1)	(15·1)
Preanal distance ..	51·5	47·5	47·0	27·0	26·0
	(82·4)	(77·9)	(78·3)	(49·1)	(49·1)
Length of head ..	4·2	5·0	5·1	6·0	7·2
	(6·7)	(8·2)	(8·5)	(10·9)	(13·6)
Length of snout ..	1·0	1·3	1·0	1·3	1·2
	(23·8)	(26·0)	(19·6)	(21·7)	(16·7)
Diameter of eye ..	0·9	0·8	0·8	0·7	0·6
	(21·4)	(16·0)	(15·7)	(11·7)	(8·3)
Total myotomes ..	124	124	124	124*	124*
Preanal myotomes ..	97	92	90	64†	58†
Predorsal myotomes ..	42	39	39	15†	12†

* Vertebræ without urostyle. † Vertebræ.

than in *M. macrura* and the values are 40·4% and 30·4% respectively. *M. macrura* also shows marked elongation of the head and snout while this is only moderate in the case of *M. tile*. Unlike the condition found in the metamorphosis of the other leptocephali where the size of the eye remains more or less constant, the metamorphosis of these two murænid leptocephali is peculiar in showing the actual reduction in the size of the eye. The eye becomes smaller in the successive stages in both the species and the smallest eye is seen in the elver. The most noticeable difference in the metamorphosis of the two leptocephali is in regard to the migration of the anus. In the case of *M. macrura* the anterior shifting of the anus takes place rather late during the metamorphosis and is seen in Stage IV only. *M. tile* shows the normal condition where the anal migration, even though seen in the growing stage itself, continues in the successive stages of metamorphosis. The elvers also show differences in the pigmentation which no doubt depends on the adult colouration. In both the species the adult pigmentation is seen to develop in Stage IV and the elver of *M. macrura* is uniformly brownish-black while that of *M. tile* is very light and the pigmentation is confined to the dorsal margin of the body and the lateral lines.

ELVERS

The different elvers of the eels whose metamorphosis is known, show certain characteristic features, which depict to some extent, the condition found in the adult eels. Among the 6 species, the elver of *Uroconger lepturus* is long and measures 88 mm. while that of *Muræna tile* is very short and measures only 53 mm. (Table III). But the latter elver shows the maximum height of 7·7% while it is as low as 3·3% in *Muræsox talabon* the head of which is higher than the body. Even though the different elvers show more or less uniform head length, the snout length shows variations and it measures 30·6% in *Muræsox cinereus* while it is only 14·4% in *Muræna macrura*. The diameter of the eye also shows slight variation and the maximum of 12·0% is seen in *U. lepturus* while the minimum of 7·8% is seen in *M. talabon* and *M. macrura*. The predorsal distance is short in all the elvers and in some it is shorter than the length of the head while the preanal distance in all the elvers is about a third of the length excepting in *M. macrura* and *M. tile* where it forms nearly half the length of the elver.

The elver of *M. macrura* is uniformly brownish-black and that of *M. cinereus* also shows a similar colouration, but the ventral side of the head and abdomen are unpigmented. The elver of *U. lepturus* is uniformly brown in colour with a black border for the vertical fins while those of the other species show only a light colouration.

TABLE III

Measurements of the different elvers

	Muraenesox talabonoides	*Muraenesox talabon*	*Muraenesox cinereus*	*Uroconger lepturus*	*Muraena macrura*	*Muraena tile*
Total length ..	78·5	84·5	62·0	88·0	69·0	53·0
Maximum height ..	3·0 (3·8)	2·8 (3·3)	4·5 (7·3)	3·8 (4·3)	4·5 (6·5)	4·1 (7·7)
Predorsal distance ..	9·3 (11·8)	10·5 (12·4)	8·5 (13·7)	11·5 (13·1)	8·0 (11·6)	8·0 (15·1)
Preanal distance ..	26·5 (33·8)	28·8 (34·1)	21·0 (33·9)	27·5 (31·3)	32·0 (46·4)	26·0 (49·1)
Length of head ..	10·0 (12·7)	11·5 (13·6)	8·5 (13·7)	10·8 (12·3)	9·0 (13·0)	7·2 (13·6)
Length of snout ..	2·5 (25·0)	2·9 (25·2)	2·6 (30·6)	2·8 (25·9)	1·3 (14·4)	1·2 (16·7)
Diameter of eye ..	0·9 (9·0)	0·9 (7·8)	0·9 (10·6)	1·3 (12·0)	0·7 (7·8)	0·6 (8·3)
Preanal vertebræ ..	42	48	43	44	103	58

Schmidt (1912) suggested the broad division of eels into two groups; one which spawns over great depths and produce specialised larvæ with a long alimentary canal and the other which spawns in relatively shallower waters with larvæ possessing short alimentary canal. Bertin (1926) while discussing the anal migration in leptocephali suggested the formula $(a_2 - a_1)/t \times 100$, where 'a_2' and 'a_1' are the preanal myotomes and preanal vertebræ before and after anal migration respectively and 't' the total number of myotomes or vertebræ for the species. Gopinath (1950) applied this formula for the leptocephali described by him for determining the spawning grounds of the adults as suggested by Schmidt and stated that " there are two distinct groups, one with a high percentage amplitude value consisting of *M. cinereus*, *C. anago* and E, and another group with a low percentage value consisting of A, C and F. Probably the first group of *M. cinereus*, *C. anago* and E may belong to eels which spawn far from the coast, while A, C and F might belong to species which spawn in shallow waters". It is not clear how Gopinath managed to calculate the percentage amplitude of the 4 unidentified leptocephali also without a knowledge of the preanal vertebral number of the elver or the adult. The percentage amplitude of anal migration of all

the leptocephali whose metamorphosis has been worked out are given in Table IV. It is seen that the anal migration varies from 8% of the total number of myotomes in *Muræna macrura* to 36% in *Uroconger lepturus* while

TABLE IV

Percentage amplitude of anal migration

Species	Preanal myotomes in lepto-cephalus	Preanal vertebræ in elver	Total myotomes in lepto-cephalus	Percentage amplitude of anal migration
Muræsox cinereus ..	78	43	138	25
Muræsox talabonoides ..	92	42	149	34
Muræsox talabon ..	80	48	150	21
Uroconger lepturus ..	122	44	218	36
Muræna macrura ..	120	103	216	8
Muræna tile ..	97	58	124	31

the others record a fairly high value, which in some is above the value of 26% found in the American eel, *Anguilla rostrata*. Further it may be pointed out that the time taken for metamorphosis of the leptocephali of *Muræsox cinereus* with a value of 26% and *M. macrura* with a value of 8% is about 10 days under laboratory conditions suggesting that the time taken for the vent to assume the adult position is about the same irrespective of its position in the leptocephali of Indian waters. Therefore, the differences in the percentage amplitude of anal migration are not useful in indicating the breeding grounds of marine eels living under tropical conditions. However, judging from the paucity of eggs, preleptocephaline stages, growing and even toothed leptocephali in the coastal waters, it is considered likely that the breeding grounds of the marine eels of Indian waters are situated away from the coast and the full-grown leptocephali generally enter the foreshore waters in the edentulous condition, where the metamorphosis is completed in a very short time.

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THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part I. The Mechanism of Perception

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1. Introduction

Light, colour and vision play a role of fundamental importance in human life and much has been written about them from diverse points of view. Nevertheless, we do not find in the literature of the subject an acceptable answer to such a simple question as the following: How and why is it possible for us to distinguish by sight the different radiations emitted by a light source, such as the mercury arc for example, and name the colours we perceive? It is no answer to such a question to state that these radiations differ from each other in wavelength. While wave-optics enables us to understand why it is possible for well-defined images of external objects to be formed on the retinæ of our eyes, the mechanism by which the incidence of the light on the retinæ is communicated to the cerebral centres and its relation to the sensations which we perceive are subjects that stand completely outside the scope of the wave-theory of light. We have therefore of necessity to seek for an answer to our questions on the basis of the alternative approach provided by the quantum theory of radiation.

If a beam of monochromatic light falls upon a matt white surface, the illuminated object as seen by our eyes exhibits two distinct subjective features, *viz.*, its brightness or luminosity and its hue or colour. From a physical point of view, the light falling on the surface has two distinct and definable properties, firstly, the radiant energy incident on it per unit of area per unit of time, and secondly, the magnitude of the quanta or units of light energy comprised in the beam. We are therefore led to associate the two characters of subjective perception respectively with the two physical attributes of the light, in other words, luminosity with energy-flux and colour with the magnitude of the individual light-quanta, and to infer that our eyes perceive colour by reason of the fact that light energy appears in discrete

quanta and that these quanta are of different magnitude for the different rays in the spectrum. That we are justified in thus associating the sensation of colour with the magnitude of the light-quanta is reinforced by the consideration that for light to be perceived it must first be absorbed by the visual receptor and such absorption necessarily occurs in complete quanta.

We have begun by considering the case of monochromatic light for the reason that it is the simplest case: monochromatic light does not admit of any further analysis. Only then it becomes possible to indicate the correlation between the physical properties of light and our sensory perceptions of the same in an unequivocal fashion. The sensations which result from the superposition of light of different colours or of different spectral composition present problems of greater complexity. They are no doubt of scientific interest and of practical importance, but we can scarcely hope that their study would lead us to an understanding of the fundamental aspects of the perception of light and colour. On the other hand, as is well known, they have led in the past to controversies and discussions of an unprofitable nature on various questions, of which it is sufficient to mention the two following as examples: Are there only three primary colours or a larger number? Which of the colours we perceive should be regarded as fundamental and which others as derived therefrom?

2. THE DUPLICITY OF HUMAN VISION

Our eyes are capable of functioning and serving us usefully over an enormous range of intensities. It has been estimated that the illumination of horizontal ground by the night-sky without moon is only about a hundred-millionth part of that received in direct sunlight, and yet it suffices for finding our way and keeping to the path provided our eyes are well adapted to darkness. That the mechanisms which function and enable us to perceive light are identical at both ends of this vast range of illumination is scarcely to be believed. Indeed, as is well known, the character of the sensations evoked is not the same in the two cases. In night-vision our eyes perceive differences in luminosity but their appreciation of colour is obviously imperfect. There are also other features which distinguish day-vision from night-vision, but we shall not pause here to discuss them.

In the year 1866, the retinologist Max Schultze announced a conclusion to which he had been led by an extended series of investigations in comparative ocular histology. He noted that the retinæ of nocturnal vertebrates had many rods, and few cones or even none, while diurnal species had retinæ with many cones which might even lack rods entirely. Thus,

arose the idea that the cones are the receptors for daylight-vision and the rods for night-vision. That two types of vision exist and must be distinguished is very generally recognized, and they have indeed been given special names, *viz.*, photopic and scotopic vision respectively. In the literature of the subject it is also generally assumed that photopic vision is mediated by the cones and scotopic vision by the rods in the retinæ. But this assignment of the two types of vision to two sets of receptors in the retina does not really take us far. We have to ask ourselves whether the physical and physiological processes are the same in the two types of vision or whether they are different and if so, in what respects.

In night-vision, our eyes have to serve us at the very lowest levels of illumination and a supersensitive receptor is clearly necessary for their successful functioning in such circumstances. On the other hand, in daylight vision, the levels of illumination are higher; indeed, the quantity of light available in day-vision is, judging by the standards obtaining in night-vision, enormously larger than is adequate for its effective perception. Hence, a relatively insensitive receptor is what is actually needed. A further aspect of the matter that should be taken note of is that our eyes have to serve us both at very low and at very high levels of illumination, though, of course, not simultaneously. It would evidently be desirable that the supersensitive mechanism of night-vision is put out of action when the insensitive mechanism needed for daylight-vision is functioning; it would, of course, then be necessary that the apparatus of night-vision is restored and put back into action when it is actually needed. No such switching off and on is needed in the case of daylight-vision.

The foregoing remarks make it clear that the mechanisms of photopic and of scotopic vision would differ in certain respects. The exact nature of such difference is however best discussed at a later stage after the actual facts regarding the photopic and scotopic perception of light have been reviewed.

3. The Facts of Colour Perception

From our present point of view, the most important facts concerning colour and daylight-vision are the relationships which exist between the subjectively perceived sensations of luminosity and colour and their physical counterparts, *viz.*, the total energy-flux and the magnitude of the energy-quanta appearing in different parts of the visible spectrum. We may first briefly recall the distribution of colour in the spectrum. The colours observed in it differ so obviously from each other that common usage finds it convenient to give them different names to aid us in recalling them to our

minds, *viz.*, red, orange, yellow, green, blue and violet. The ranges over which these designations are applied vary with the individuals concerned, and only a kind of general average can therefore be regarded as possessing any significance. Expressed in wave-numbers and therefore directly related to the magnitude of the energy-quanta, these ranges are, respectively, Red (13,000–16,000), Orange (16,000–17,000), Yellow (17,000–17,600), Green (17,600–20,200), Blue (20,200–23,000) and Violet (23,000–26,000). It is noteworthy that while the ranges in wave-numbers covered by the designations red, green, blue and violet are all of the same order of magnitude, the colours designated as orange and yellow cover much smaller ranges.

We shall return presently to a more detailed discussion of the colour sequence observed in the spectrum, and proceed meanwhile to consider the relation between the subjectively perceived brightness in different parts of the spectrum and the physically measurable energy-distribution in it. The essential features of this relationship can be established in a very simple manner by holding a diffraction grating in front of the eye and viewing the straight filament of a tungsten lamp, the current flowing through which is controlled by a rheostat. Observations of the first-order spectrum into which the luminous filament appears drawn out show that the distribution of luminosity in it is quite different from the energy-distribution in thermal black-body radiation as given by the Planck formula. According to the latter formula, the wavelength of maximum energy in the spectrum lies in the remote infra-red when the source has a temperature of 500° K., and though it moves towards the red end of the visible spectrum as the temperature is raised, it remains outside the visible spectrum even at as high a temperature as 3,000° K. On the other hand, the maximum luminosity as visually observed appears well within the spectrum even when the temperature of the filament is so low that it glows only feebly with a red colour. As the temperature of the filament is raised and it becomes brilliantly incandescent, the point of maximum luminosity in the spectrum moves swiftly towards shorter wavelengths and finally reaches a point in the greenish-yellow region of the spectrum at about 5600 angstroms beyond which it ceases to move any further. This is also the wavelength of maximum visual intensity in the spectrum of direct sunlight. In the spectrum of the light of the blue sky, in which the energy-distribution relative to sunlight is radically altered, the position of the maximum visual intensity is nevertheless not appreciably different. It is evident from these facts that it is a characteristic of daylight-vision that the eye has a highly pronounced maximum of visual sensitivity at about 5600 angstroms on either side of which the visibility of radiation falls off rapidly. It is also evident from the observations that

such falling off of visibility is less rapid towards the red end of the spectrum than towards the violet.

The features referred to above find quantitative expression in the "relative luminous efficiency" curves for monochromatic radiations appearing in the visible spectrum which have been determined by several investigators. The two methods most often used for this purpose are the so-called step-by-step method and the flicker method. In the former method, the difficulties of heterochromatic photometry are minimised by dividing up the spectrum into a fairly large number of strips (twenty or more) and successively comparing the intensities of adjoining strips. In the other method, the luminosity of monochromatic radiation is determined by the method of comparison at the rate of 10–20 times per second against a constant source of white light, the intensity of the monochromatic light being varied until the flicker experienced is a minimum. Differences are noticed between the determinations made by different observers, but by averaging the results obtained with a large number of observers, a luminous efficiency curve is obtained which can be regarded as a property of normal human vision. The results obtained by the two different methods are about the same, though some discrepancies are noticeable. This is shown by the two curves reproduced as Fig. 1 below exhibiting the results obtained by Gibson and Tyndall with the step-by-step method, and by Coblenz and Emerson with the flicker method.

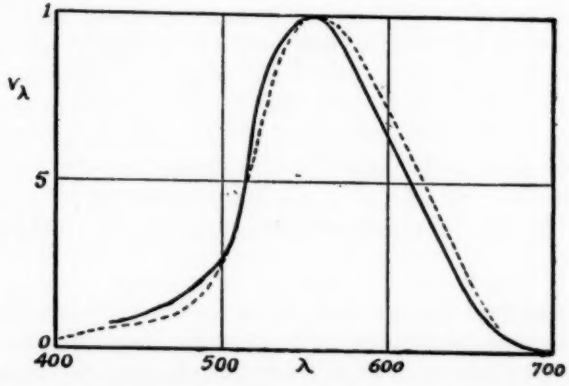

FIG. 1. Relative luminous efficiency curves by the step-by-step method (——) and the flicker method (– – – –).

We now return to the colours exhibited by monochromatic light. Earlier, it was indicated that the varying colours of the spectrum represent

our subjective perception of the physical fact that the energy-quanta are different in its different regions. On this basis, we should expect the colours of the spectrum to form a continuous sequence, in other words that the spectrum can be divided into a great many parts or sections of which the colour can be perceived to be different. That is indeed actually the case. The number of distinct patches in the spectrum which when set side by side cannot be matched by any alteration in relative brightness has been estimated as about 250. If all the sections into which the spectrum may thus be divided were of equal width on a frequency scale, the smallest spectral shift giving rise to an observable change in colour would be about 50 wave-numbers. If this figure were accepted, it would mean that the human eye possesses a most remarkable degree of sensitivity to differences in colour and that it is capable of appreciating a difference less than one-half of one per cent. in the magnitude of the light quantum received and absorbed by the retina as a difference in colour of the radiation.

It should be remarked, however, that the minimum spectral shift needed for a noticeable change of colour is not the same at all parts of the spectrum. It exhibits very significant variations, being in some parts smaller and in other parts larger than the figure stated above. Numerous investigations have been made in the past to determine the minimum in various parts of the spectrum and when the results reported are compared with each other, one finds a general agreement regarding certain features, *viz.*, that in the region around 4900 angstroms and also in the region around 5900 angstroms, the minimum has quite small values, being respectively about 9 angstroms and 11 angstroms, and that in the intermediate region, *viz.*, around 5400 angstroms, it is distinctly larger, being about 21 angstroms. Expressed in wave-numbers, these same figures are, respectively, 38 and 26 and 62 wave-numbers as against the averaged figures of 50 stated above. At wavelengths less than 4900 and greater than 5900 angstroms the determinations show that the minimum shift of wavelength increases rapidly. In these regions, however, the quantitative results of different investigators differ rather widely from each other.

The method by which the data of the kind referred are obtainable may be briefly described as follows: The observer views the two halves of a photometric field illuminated respectively by light from two adjacent regions in the spectrum of which the separation can be varied. The relative brightness of the two halves of the field is adjusted to equality by the use of a rotating sectored disk or by some other device. If, when so matched, the two parts of the field appear definitely different, the spectral shift giving

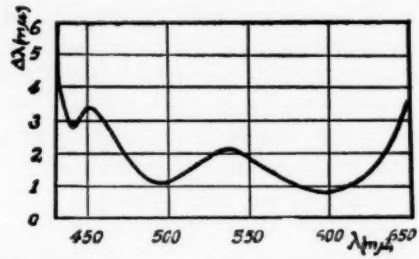

FIG. 2. Hue discrimination curve in the spectrum, according to Wright and Pitt.

rise to the difference represents the least perceptible to the eye as a difference in colour. Figure 2 above exhibits "the hue discrimination curve" in the spectrum obtained in this manner and reported by two observers. The abscissæ in the curves are the wavelengths of monochromatic light in millimicrons, while the ordinates are the smallest shifts of wavelength (also in millimicrons) giving rise to an observable difference in colour. The figure exhibits the features already referred to above, and another not there mentioned, *viz.*, the existence of a marked dip in the hue discrimination curve at about 4400 angstroms. Most of the published investigations report this feature and there is no doubt that it represents a well-established result.

Quite apart from the results of specific measurements made by the methods referred to above, it should be stated that a simple inspection of the spectrum by the unaided eye indicates that the colour sequence in the spectrum changes rapidly in certain regions, less rapidly in others and much less rapidly in certain others. An observer with normal colour vision will notice that the change of the blue to the green of the spectrum takes place within a relatively small range of wavelengths between, say, 4900 and 5000 angstroms. Likewise, he will notice that the transition from the yellow to the orange of the spectrum takes place between, say, 5900 and 6000 angstroms. On the other hand, the transition from green to yellow is continuous, so much so that it is difficult to locate with any exactness the boundary between these two colours. The boundary between the blue and violet regions of the spectrum is placed in very different positions by different observers and indeed, it would seem that Newton was not altogether without justification when he put an additional colour "indigo" between blue and violet.

4. THE MECHANISM OF PHOTOPIC VISION

We now proceed to consider the significance of the facts regarding colour perception set forth above. For the reasons already explained, we

associate the sensation of luminosity with the physical intensity or energy-flux, and the sensation of colour with the magnitude of the energy-quanta comprised in the radiation. Both the luminosity and colour of monochromatic radiation admit of study by appropriate physical methods which lead to quantitative results. These results do not contradict the physical interpretations proposed and they accordingly require to be further examined and explained. In other words, we have to answer various questions concerning them. In the first place, why does the luminous efficiency of monochromatic radiation vary in the manner observed and exhibit a maximum around 5600 angstroms? What is the factor which limits the power of the eye to discriminate differences in colour in the different parts of the spectrum? Why is it as great as it actually is in some parts, and why it is smaller in others?

The human eye has often been compared with a photographic camera. There are indeed some resemblances; in both cases we have a light-tight box covered inside with dark pigment to absorb unwanted light, a shutter to exclude light when desired and an adjustable diaphragm to regulate its admission when necessary. In both cases also, we have an optical system with front and rear components capable of adjustment so as to form a focussed image at the rear of the box. But to stress these structural similarities between a photographic camera and the human eye is liable to make us forget that the basic purposes which they are intended to serve are fundamentally dissimilar. A photographic camera serves to make a record of the light image on the sensitive film which can be developed and preserved. On the other hand, the retina of the eye serves both as a receiver and as a transmitter to the cerebrum of the energy of light falling on it. Whereas a photographic film can be used only once, the retina of the human eye is always ready for use. Indeed, vision would be useless to us if it did not carry with it the ability to view objects in different directions in quick succession one after another.

It is self-evident that light which merely passes through the retina and is absorbed by the pigmentary epithelium behind it would be ineffective in vision. In other words, the absorption of light by pigments present within the retina itself is the first and necessary step for vision to be possible. But it is equally evident that unless such absorption goes hand in hand with a retransmission of the absorbed energy to the cerebral centres through the optic nerves, vision cannot result. It should also be remarked that since the colour perceived is determined by the magnitude of the energy-quanta comprised in the radiation, we have to assume that each quantum of energy

absorbed is transferred immediately to the neurons without addition or subtraction. It would not be possible otherwise to explain the remarkable precision actually exhibited by our sense of colour discrimination. Merely as an example of such sensitivity, it may be mentioned that the two components of the sodium doublet whose wavelengths are 5890 and 5896 angstroms can be perceived to be of different colours in appropriate circumstances.

The facts of colour perception thus lead us to a quite simple and definite picture of the functioning of the retina in photopic or day-vision. We postulate that the light-sensitive receptors in the retina contain certain pigments which possess the power to transfer the light-energy absorbed by them to the neurons and return to their state of lowest energy. The known facts regarding the propagation of nervous disturbances and the production of electrical potentials by the infall of light on our eyes suggest that the energy transferred from the pigments assumes the form of an electrical disturbance in the receptors which is conducted through the optic nerves and on reaching the cerebral centres there excites the sensations we perceive. But we shall not pursue this aspect of the matter any further here.

5. The Characters of Scotopic Vision

We now proceed to consider the actual facts regarding the perception of light at low levels of illumination, also referred to as scotopic vision. Earlier in this memoir it was suggested that the duplicity of human vision calls for a mechanism which could switch off the apparatus of scotopic vision when there is ample illumination and photopic vision can therefore function, and would switch it on again when the illumination is very feeble and photopic vision ceases to be possible. That such a mechanism is actually provided is evident from our own sensations when we move from sunshine into a dark chamber and *vice-versa*. The switching-on process is the slower of the two, since it is necessary to remain in the dark for at least thirty minutes for our eyes to develop the full sensitivity characteristic of scotopic vision. The switching-off process is somewhat quicker, but its duration is found to depend on the intensity of the light to which the retina is exposed.

Earlier, it was mentioned that in scotopic vision, the colour sense is imperfect. Such imperfection is due largely to the circumstance that the great sensitivity to light characteristic of scotopic vision does not extend to the parts of the spectrum which are both luminous and colourful, *viz.*, the red and orange regions. Indeed, when any object of a scarlet-red colour

is viewed in a darkened room, it appears quite black. The characteristic features of scotopic vision are however best appreciated by studying the appearance of a continuous spectrum in scotopic conditions. A simple and convenient way of doing this is for the observer in a dark room to examine sky-light entering the room through a long vertical slit through a glass diffraction grating held in front of his eye. If the observations are made in day-time, the first-order diffraction spectrum of the slit then seen exhibits the familiar features characteristic of photopic vision. If, on the other hand, the observation is made at night when the sky is lit by moonlight (or in the absence of the moon by the lights of the city), the spectrum appears greatly shortened by the absence of all wavelengths greater than about 6000 angstroms. By making the observations during the twilight hours (evening or morning), the progressive change from the photopic to the scotopic spectrum or *vice-versa* can be readily followed. The change-over can be hastened or slowed down by the simple method of narrowing or widening the slit through which sky-light enters the dark room, or by the observer moving away from or towards the slit. In either case, large changes in the visual intensity of the spectrum result and the entire series of changes from the photopic to the scotopic conditions can be quickly and conveniently followed. Indeed, one can go down to the stage in which the intensity of the spectrum approaches the threshold of vision.

A characteristic feature of the spectrum seen under scotopic conditions is that the wavelength of maximum visual intensity shifts towards the blue as compared with photopic vision. Quantitative determinations of the relative luminous efficiency curve at low levels of illumination have been made by several observers, the method used being that of direct photometric comparison against a white field of low luminosity. A typical set of results is shown graphically in Fig. 3. It will be seen that the relative luminous efficiency becomes negligible at wavelengths greater than about 6000 angstroms, while its maximum appears at about 5100 as against 5600 angstroms in photopic vision.

A question of special interest is whether the spectrum seen under scotopic conditions exhibits the colour sequence seen in photopic vision (apart from the disappearance of the longer wavelengths) or whether the scotopic spectrum is achromatic as is often stated. Observations made by the methods described above gave a clear answer to the issue here raised. Scotopic vision covers a wide range of intensities differing at its two ends by a large factor; whether in any particular case, we are observing the spectrum under scotopic conditions is indicated by the relative luminosity of the regions

around 5000 and 6000 angstroms, the latter being negligible in scotopic conditions, while in photopic conditions, the latter is much more intense than the former. While the scotopic spectrum exhibits no visible colour anywhere at the lowest levels of intensity, this ceases to be the case at greater intensities. A greenish tinge becomes visible around the region of maximum luminosity; this is more obvious at greater intensities, and the differences in colour between the centre of the spectrum and its two ends are also then perceptible. As we approach the upper limits of scotopic vision, the colours observed become more vivid, but remain distinctly weaker and less saturated than in photopic vision.

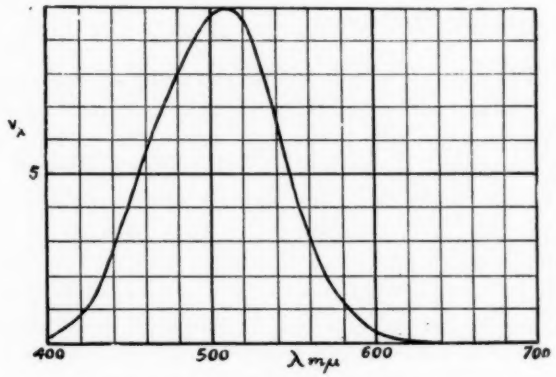

FIG. 3. Relative luminous efficiency in scotopic vision.

6. THE MECHANISM OF SCOTOPIC VISION

In their fundamental aspects, scotopic and photopic vision resemble each other closely. It is just as easy to view different objects one after another in quick succession at low levels of illumination as at higher levels and indeed one is then less troubled by after-images, glare and other disturbances. Visual photometry is possible in scotopic as in photopic vision and indeed in some respects easier, as for instance in heterochromatic photometry. If scotopic vision had been entirely achromatic at all levels of illumination, this feature would differentiate it fundamentally from photopic vision. But, as we have seen, such is not the case. The colour sense is actually present at the higher levels of illumination falling within the scotopic range and the colours then perceived, though less vivid, do not otherwise differ from those seen in photopic conditions.

In view of what has been stated, one would be justified in assuming that in their basic aspects, the mechanisms of scotopic and photopic vision

are similar, in other words that in either case, the retinal pigments function by a simple transference of the light energy absorbed by them to the receptors, followed by a return to their normal energy state. The differences between the characters of photopic and scotopic vision described earlier do not appear to be inconsistent with such an assumption. That the relative luminous efficiency curve for scotopic vision differs from that determined for photopic vision is readily understood on the basis that the retinal pigments which function in the two cases are different. That scotopic vision goes down to lower levels of illumination also becomes intelligible on the basis that the retinal pigment which functions is present in substantial quantities and can therefore trap a larger proportion of the incident energy quanta and pass it on to the receptors.

It now remains to explain how the switching-off and switching-on of scotopic vision take place respectively when the observer moves from low levels of illumination to high levels and *vice-versa*. It may justifiably be inferred from the actual facts of observation that when the observer has been long enough in bright light, the pigment which mediates scotopic vision has been transformed by the incidence of such light on his retina into a different substance which cannot so function; *vice-versa*, when the observer remains in darkness long enough, the pigment is regenerated in some fashion. The switch-off would proceed at a rate proportional to the intensity of light to which the retina is exposed and would be the quicker, the more intense such illumination is. The regeneration of the pigment, on the other hand, would occur in the absence of light or at very low levels of illumination and its rate would be determined by purely chemical or biochemical factors. The destruction of the pigment by bright light and its regeneration in the dark here contemplated are merely auxiliary processes which make it possible for human vision to function efficiently both at high and at low levels of illumination. There is no need and indeed no justification for assuming that they form an essential part of the actual mechanism of scotopic vision. Neither would photopic vision demand the destruction and regeneration of the visual pigments which function in it as an essential part of the visual mechanism.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part II. The Visual Pigments

By Sir C. V. Raman

(*Memoir No.* 125 *of the Raman Research Institute, Bangalore-6*)

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1. Introduction

In the first part of this memoir, the view has been put forward that our perception of light and colour is made possible by the presence in the retina of certain pigments which possess the power to absorb light and to transfer the energy thus absorbed immediately to the sensory receptors, thereby enabling us to perceive the absorbed energy as light. The identification of the visual pigments which perform these functions, in other words the determination of their chemical nature and a knowledge of their spectroscopic behaviour and distribution in the retina are the foundations on which any explanation of the facts of experience regarding vision must rest. We shall address ourselves in this part of the memoir to the problem which thus confronts us.

2. Viewing the Retina in Action

Any observer endowed with normal colour vision can perform the experiments now to be described. They do not require elaborate equipment and enable him to see his own retina in the act of functioning and observe its response to light in different parts of spectrum. From the results of the experiments, it is possible to infer the number and the distribution in the retina as well as the spectroscopic behaviour of the visual pigments which enable us to perceive light and colour.

The technique of the observations is quite simple. The observer sits facing a brightly illuminated white screen at a convenient distance from it and views the screen through a colour filter, either monocularly or binocularly as he may find best suits him. After allowing a sufficient time for the vision to adapt itself to the effects of the colour filter, the observer fixes his vision on some particular point on the screen and suddenly removes the filter. He will then recognize on the screen a highly enlarged projection of the macular region of his own retina, including especially the fovea

centralis and the foveola at its midpoint. The details of the picture seen and the colours it exhibits depend very much on the filter employed. The picture soon fades away but it can be restored by putting back the filter and removing it again at short intervals of time. The picture can then be examined more closely.

The appearance of an image of the observer's own retina on the screen can be explained in general terms in the following manner. The colour filter employed cuts out or enfeebles certain parts of the spectrum while transmitting the other parts of it freely. As a consequence of the removal of the filter, the spectral components which are cut out or enfeebled by it are restored and suddenly illuminate the retina. Localised sensations are then excited, determined by the luminous efficiency of these spectral components and by the response to them of the receptors located in each element of area of the retina. The sensations thus excited manifest themselves to the sensory mechanism of the eye as an image of the retina which appears projected on the screen, each element of area exhibiting its own response to the illumination suddenly falling on it.

Before we proceed to describe and comment on the results observed with individual filters, it may be useful to mention a few particulars requiring attention in the experiments. It is necessary that the illumination of the screen should be adequate and that it should be as nearly uniform as possible. The observations should therefore be made by daylight, the screen being placed facing the windows in a well-lighted room and the observer should sit facing the screen and with his back to the windows. A projection screen of the well-known kind which is plastered over with tiny glass spheres is very suitable. A medium-sized screen, say 170 cm. by 125 cm., is adequate and a convenient distance from the observer to a screen of that size is 350 cm. It is possible to use colour filters of different kinds, as for example glass cells of sufficient size containing absorbing solutions or plates of coloured glass. However, for the studies now under consideration, the most convenient filters are those which can be prepared by staining gelatine films on glass (of the kind used in photography) with a water-soluble dye. By regulating the strength of the solution in which the plates are dipped and the period of immersion before they are taken out and allowed to dry, the depth of the staining can be controlled over a wide range. The filters should be held close to the eye so as to exclude all light except that passing through it.

3. DESCRIPTION OF THE EFFECTS OBSERVED

Quite spectacular effects are observed when the filter employed is a gelatine film on glass stained lightly with methyl-violet. The filter appears

a purplish-blue by transmitted light. Holding it before the eye for a few seconds and then removing it, the observer sees on the screen an enormously magnified image of his own fovea as a disk of light which is green in colour, and at the centre of it a bright spot of the same hue which is the pit or depression in the fovea known as the foveola. If the filter is held for a somewhat longer time before it is removed, the fovea is much brighter and then appears surrounded by a halo of golden-yellow hue some five or six times larger in diameter but less luminous than the fovea itself. The fovea with the foveola at its centre appears at the point on the screen at which the observer has fixed his vision before removing the filter. If he shifts his gaze, they also move, thereby showing clearly that what is seen on the screen is a projected image of the observer's own retina. (See Colour Plate, Fig. I.)

Spectroscopic examination shows that the filter lightly stained by methyl-violet has a strong absorption band in the orange, a weaker one in the green, and a very weak absorption in the region between them. When more heavily stained, the absorption becomes complete in all these regions and extends further into the green. A film stained heavily so that it cuts out the green completely but transmits the red and the blue of the spectrum appears of a deep purple colour. The use of such a heavily stained filter is however of no particular advantage, and on the other hand makes the phenomena rather less spectacular.

A noteworthy fact that emerges from the studies is that a filter whose absorption spectrum appears *exclusively* in the green part of the spectrum, in other words between 4900 and 5600 angstroms, does *not* give any observable effects of the kind referred to above. Several colour filters of this kind are available, amonsgt which may be mentioned very dilute solutions of potassium permanganate in water, or of iodine in carbon tetrachloride. More convenient, however, are gelatine films stained lightly by a suitable dye as, for example, rhodamine or eosine, so as to enfeeble or cut out the green region without affecting the rest of the spectrum. Holding such a filter in front of the eye for a little while and then removing it, the screen presents the same appearance as before the filter is interposed. Prolonging the period for which the filter is held before the eye prior to its removal makes no noticeable difference.

Though, as stated above, colour filters that absorb only the green part of the spectrum do not give rise to the effects under consideration, it is clear from the example of the methyl-violet filter that absorption in the green

region can co-operate with absorption in adjoining regions of greater wavelengths and enable them to be observed. We may therefore conveniently divide the spectrum into three regions which we may denote as A, B and C and classify the filters and the effects they produce by reference to the regions of the spectrum in which their absorption appears. The violet-blue region of the spectrum is denoted by A, the green by B, and the yellow, orange and red may be grouped together and referred to as C.

As an illustration of the usefulness of the classification proposed in relation to the effects observed, we may here mention two cases in which very beautiful effects are observed but of a different nature. The colour filters in both cases are aqueous solutions contained in glass cells of equal thickness, five centimeters in each case. One solution is that of nickel chloride and the other of the dye-stuff lissamine-green sufficiently diluted so as to transmit plenty of light. The nickel chloride solution is completely transparent in the green, and examination by a pocket spectroscope shows that it also allows the adjoining part of the blue to pass through, but cuts out the red, orange and yellow more or less completely. It thus belongs to the class of filter which we denote as AC. The solution of lissamine-green appears blue-green by transmitted light and while resembling the nickel chloride in allowing part of the blue to come through, it cuts outs the red, orange and yellow completely and also part of the green. It thus belongs to the class ABC.

Holding the nickel chloride solution before the eye for a few seconds and then removing it, a brilliant rose-red glow is seen to cover the entire screen; at the centre of the field and as a very inconspicuous feature appears a small circular area which appears of a different hue and brightness from the rest of the field. On the other hand, when the lissamine-green solution is used for the observations, a highly magnified image of the fovea, bright yellow in colour and more luminous than the rest of the field, and the foveola at its centre are the most conspicuous features which the observer sees on the screen. A reddish glow covers the entire screen, but around the foveal image appears a halo of indefinite hue. (See Colour Plate, Fig. II.)

The filters of the species designated as A and as AB transmit the red, orange and yellow regions of the spectrum perfectly but cut off the shorter wavelengths to varying extents. As their cut-off in the spectrum moves from the extreme violet into the green and then further, the colour of the transmitted light progressively alters from the palest yellow to the deepest orange. All filters of the kind mentioned exhibit effects having certain

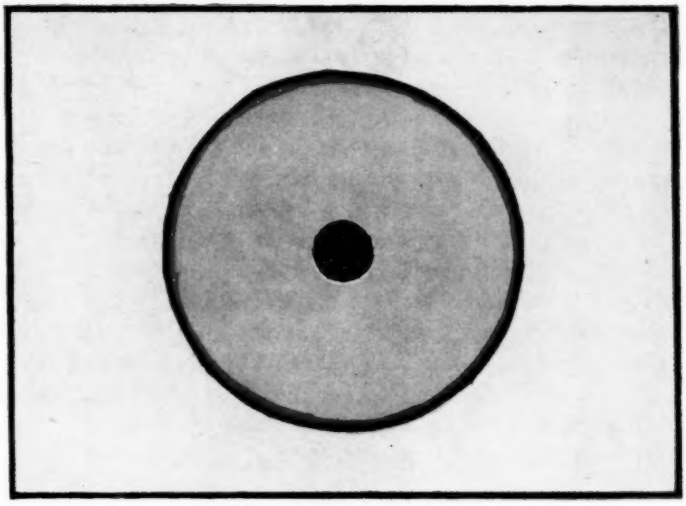

Fig. 1 Methyl Violet Filter

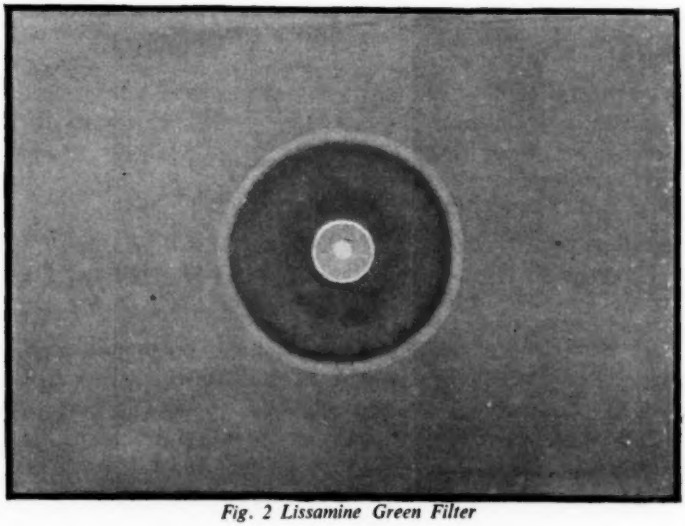

Fig. 2 Lissamine Green Filter

Pictures of Retina seen by the Filter Technique.

features in common, though differing in detail. For example, using a filter of pale yellow hue having a cut-off at 4500 angstroms, its sudden removal from before the eye after an adequate interval results in the appearance of a violet-tinted glow which covers the entire screen. Neither the fovea nor the foveola nor any halo surrounding them can be seen. But at the centre of the field in the direct line of the observer's vision, there is a hint of the glow being somewhat dimmer than elsewhere on the screen. With another filter of a deep yellow colour which has a cut-off at 5100 angstroms, similar effects are noticed, but the glow which appears covering the screen is blue in colour and much brighter than that observed with the pale yellow filter. Orange filters give effects which are similar to those seen with the deep yellow filter but are not by any means more conspicuous; on the other hand, the effects are rather less conspicuous since the glow does not display any vivid colour and also ceases more quickly to be observable. Thus, an extension of the region of absorption by the filter from section A of the spectrum into section B does not make any real difference to the results, apart from making them less easy of observation.

Gelatine filters of the classes C and AC may be prepared by staining them *very lightly* with the dyes methylene-blue and lissamine-green respectively. Holding such filters before the eye and then removing them, the entire area of the screen exhibits a glow of a colour which differs for the two species of filter, orange in one case, and rose-red in the other. But no image of the fovea or foveola appears on the screen. Only filters of the classes BC and ABC exhibit the later phenomenon. They may be prepared in great variety by staining gelatine films to any desired depth by blue or green, dyes (methylene blue and lissamine-green for example). Most blue glasses belong to the class BC and most green glasses to the class ABC. A photographic filter exhibiting a delicate green colour was however found to belong to the class AC. It cuts out the violet rays in the spectrum and visibly enfeebles the red, but has no noticeable absorption in any other part of the spectrum. Holding it before the eye and then removing it, the observer sees a beautiful purple glow covering the entire screen, while an inconspicuous feature appears at the centre of the field of vision in the shape of a small circular area where the glow is feebler than elsewhere.

Observations have been made with numerous filters of the classes BC and ABC. With every one of them, the foveal disk and the foveola at the centre are conspicuous features. It is also possible in many cases to observe a coloured halo five or six times larger in diameter than the foveal disk but

of much lower intensity superposed on the general field of illumination on the screen following the removal of the filter.

4. THE THREE VISUAL PIGMENTS

We now proceed to consider the significance of the facts of observation reported above. We have seen that the entire spectrum may be divided into three sections which we have denoted as A, B and C respectively, and that the colour filters whose absorption appears exclusively in one or another of these three behave quite differently. It is a reasonable interpretation of the facts that the retina contains three pigments which are effective as absorbers of light and mediators of vision respectively in these three sections. We shall refer to them hereafter as pigments A, B and C respectively. Pigment A functions in the blue-violet region of the spectrum, pigment B in the green and pigment C in the orange, red and yellow. The facts of observation described earlier, however, compel us to qualify this statement and to recognize that the absorption spectrum of pigment C overlaps that of pigment B in the green to an appreciable extent. It is also necessary to assume that when the incident light causes pigment C to function, this results in the simultaneous functioning of pigment B in the same region of the retina, provided the wavelength of the incident light is within the region of overlap of the absorption spectra of the two pigments.

The effects observed on the viewing screen following the removal of the filter enable us to draw some useful inferences regarding the distribution in the retina of the visual pigments which give rise to them. The position is clearest with regard to pigment A which is the mediator of vision in the blue-violet region of the spectrum. The blue or violet glow covering the entire area of the screen seen with filters of class A clearly indicates that the visual pigment of that class is distributed over an extent of the retina many times larger in area than the fovea itself.

The appearance on the viewing screen of a highly enlarged and luminous image of the fovea and of the halo encircling it clearly demands the co-operation of the two pigments B and C, since these effects are only noticed when filters of the classes BC and ABC are employed. The colours displayed by the fovea and the halo in various cases also point to the same conclusion. We are thus led to infer that pigment B is present in its maximum density in the region of the fovea and has a considerable though smaller density in an area surrounding the fovea and having five or six times its diameter. The observations further indicate that pigment C is present in association with pigment B in the areas referred to and that it is also to be

found distributed over the area of the macula well beyond the region in which the coloured haloes surrounding the fovea are observed.

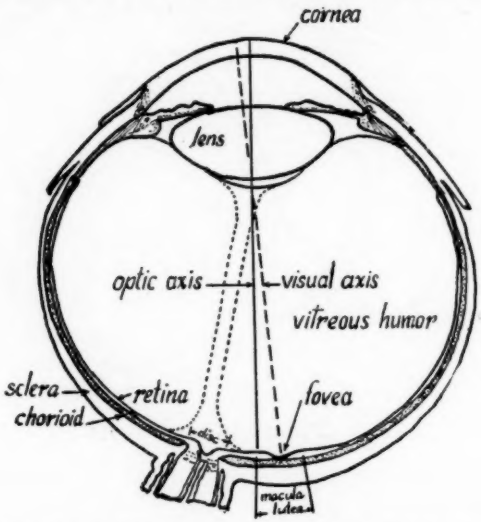

FIG. 1. Horizontal section of the right human eye.

We may reasonably look for some evidence in support of the foregoing findings in the appearance of the retina as seen in the ophthalmoscope. The fundus of the human eye is visible in that instrument by reason of the diffusion of the light incident on the retina by the retina itself and by the materials behind it. The opinion expressed in the ophthalmological treatises (and copied therefrom into other books) is that the appearance of the retina is due to the diffusion of light by the choroid coat which lies behind the retina. Between the retina and the choroid coat, however, lies the pigment epithelium, the function of which is to absorb unwanted light traversing it in either direction. The efficiency of the pigment epithelium as an absorbing screen has therefore a very great influence on the nature of the picture seen in the ophthalmoscope. This is indeed evident on a comparison between the appearance of the retina in the four cases of albinotic individuals, persons of fair complexion, and persons of dark and very dark complexions respectively. The blood-vessels in the choroid are clearly seen through the retinæ of albinotic individuals. They are not seen in the other cases, what is actually visible being the surface of the retina upon which can be distinguished the optic disk, the retinal blood-vessels and the macula. The hue exhibited by the retinal surface is orange-red in persons of fair complexion, while in the case of negroes it is brick-red.

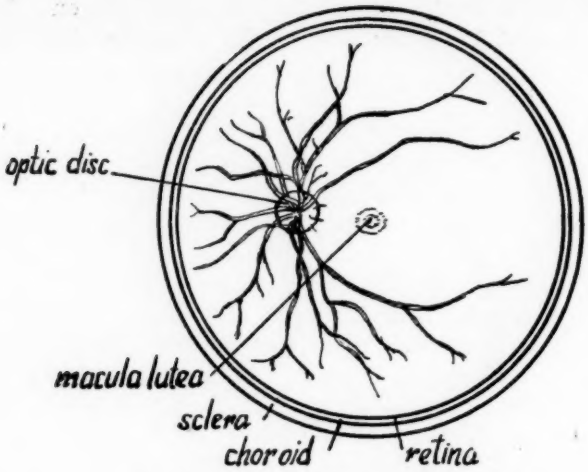

FIG. 2. Fundus of the human eye.

What we are here specially interested in is the macular region which is physiologically the most important part of the fundus. This region is devoid of any visible blood-vessels, though it is encompassed on all sides by the twigs reaching down to it from the retinal blood-supply system. A specially significant fact is that in all cases, the macular region is of a distinctly darker tint than the rest of the fundus. Further, the fovea itself exhibits a blood-red hue in all cases. These are conspicuous features in the coloured illustrations of the fundus appearing in the ophthalmological treatises. The presence of pigments which between them cover the entire spectrum would explain the deeper colouring of the macular region. The presence in and around the fovea of a pigment which exercises a powerful absorption in the green region of the spectrum would account for that area exhibiting a blood-red hue. Thus, what is actually seen of the fundus through the ophthalmoscope does not contradict the conclusions reached through the present studies, but on the other hand gives them the clearest possible support.

A further remark may be made here. Ophthalmologists not infrequently in their examination of the fundus make use of light from which the red rays have been excluded by a suitable filter. It is stated that the background of the retina then appears of a yellowish-green colour, the macula standing out as a lemon-yellow area, and that the blood-vessels running through the retina appear almost black with sharply defined outlines. That the macula is distinguishable by its colour from the rest of the

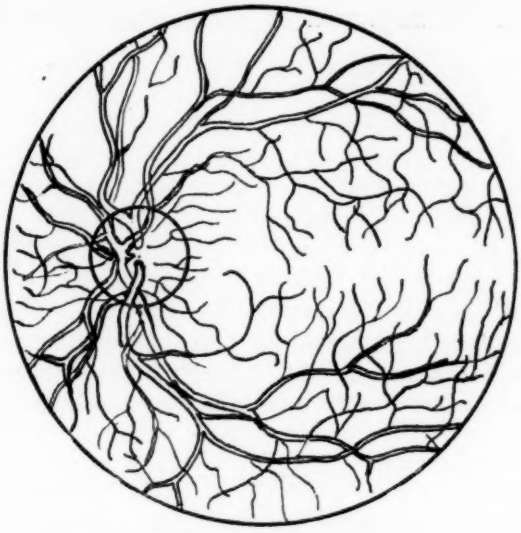

FIG. 3. Blood-vessels in the retina.

retina in these circumstances has been explained as due to presence in it of appreciable quantities of the yellow pigment which gives to the macular region its anatomical name of *macula lutea*. This yellow pigment may reasonably be identified with our pigment A, the absorption of which appears exclusively in the blue-violet region of the spectrum.

FIG. 4. Perifoveal capillaries in the retina.

5. IDENTIFICATION OF THE VISUAL PIGMENTS

We now proceed to consider the problem of determining the chemical nature of the pigments present in the retina which are the mediators of photopic vision. We have designated them above as (A), (B) and (C) and formed a general idea of the spectral ranges within which they are operative. They should be capable of existing in association with the materials which form the living substance of the retina. Their absorption spectra should appear in the regions indicated and it is necessary that when they absorb light radiations, they should also be capable of passing on the energy thus absorbed immediately to the receptors in the retina. Finally also, since we are concerned with photopic vision and therefore with high levels of illumination, it is necessary that the pigments should possess in a reasonable measure the power to resist disruption or decomposition by the action of light.

The advances in organic chemistry made of recent years have resulted in the elucidation of the structure of numerous naturally occurring colouring matters and created a great body of knowledge connecting chemical constitution with optical behaviour. A well-established result which emerges from these researches is the relation between the manifestation of colour by a substance and the presence in its molecules of unbroken chains of alternate single and double bonds. It is found that the longer the chain of such bonds is, the further into the region of the visible does the absorption spectrum of the substance extend. Many naturally occurring pigments also fall into two classes, one in which the chain of alternate single and double bonds is an extended straight line, and the other in which it forms a closed ring within the molecule. The group of substances known as the carotenoids belong to the first class, while in the second class we find various substances playing highly important biological roles, including especially heme and chlorophyll. It is interesting and significant that the green leaves of growing plants contain colouring matters belonging to both of the classes referred to. Two of them, *viz.*, β-carotene and xanthophyll belong to the first class and two others, *viz.*, chlorophyll-*a* and chlorophyll-*b* to the second.

As is well known, the pigments present in green leaves form the pathway by which organised nature finds access to solar energy. They enable the energy of solar radiation to be utilized for the synthesis from carbon dioxide and water of carbohydrates and other constituents of living matter. The mechanism of this process—known as the photosynthesis by plants—has been the topic of numerous investigations. It is recognized that chlorophyll with the strong absorption of light which it exhibits at the extreme red end

of the spectrum plays a leading role in photosynthesis and the view formerly prevailed that the other pigments present did not participate in the process. More recent studies indicate that the latter conclusion needs modification and that the carotenoid pigments also do play a part in photosynthesis. Cogent evidence is also forthcoming that the pigments in green leaves, including especially chlorophyll, act as energy-receiving and transferring agents and thus enable photosynthesis to take place. Whether they actually participate in the chemical changes which result in the formation of new compounds and if so in what manner are questions which at the present time remain in the speculative stage.

It would seem, therefore, that the pigments in green leaves and the pigments in our retinæ play somewhat similar roles in their respective fields of activity. Hence, there are good grounds for assuming that the pigments concerned are either the same or else are very similar. Four pigments are needed for vision, three for photopic and one for scotopic vision. Two of them may well be carotenoids, one for each type of vision. There is no reason, however, for assuming that all four pigments are carotenoids. On the other hand, it is most improbable that this would be the case. Pigment B plays a role in human vision of special importance as it covers the part of the spectrum having the highest luminous efficiency. One would naturally expect that such a pigment would be a product of biological activity in the human organism and not a substance carried into the body through the medium of food products. Further, it is difficult to believe that any carotenoid would possess all the properties needed for pigment B, *viz.*, an absorption spectrum lying in the region between 5000 and 6000 angstroms, a high efficiency as an energy-receiving and transferring agent and the chemical stability needed to resist disruption or decomposition under intense illumination. We are thus led to infer that pigment B belongs to the class of organic compounds which derive their colour from the presence in their molecular structure of a ring of alternating single and double bonds. That pigment C also belongs to that class is *prima facie* very probable.

Chlorophyll-*a* and chlorophyll-*b* are pyrrole pigments which are chemically very similar to each other. They owe their colour to a combination of four pyrrole nuclei joined together in a complex molecule, a special feature of importance being that a magnesium atom appears linked to the nitrogen atoms of the four pyrrole groups in the molecule. When the magnesium atom is removed by the action of acids, the solution turns brownish and ceases to exhibit the colour characteristic of chlorophyll, thus demonstrating the importance of the metallic atom in relation to its spectroscopic behaviour. When we seek for a pigment which could perform in

the retina a role analogous to that performed by chlorophyll-*a* in the green leaf, the obvious and indeed the only choice is the metallo-porphyrin compound known as ferroheme (or simply as heme). This is also a pyrrole pigment, being a compound of iron in the divalent state with one of the porphyrin group of compounds. The porphyrins contain a closed ring of eighteen bonds which are alternately single and double in their structure, and the iron atom occupies in heme a position in relation to the four nitrogens in the molecule which is the same as that of the magnesium atom in the chlorophyll structure. There is thus good reason for assuming that heme possesses the properties needed to enable it to function very efficiently as a visual pigment in the retina.

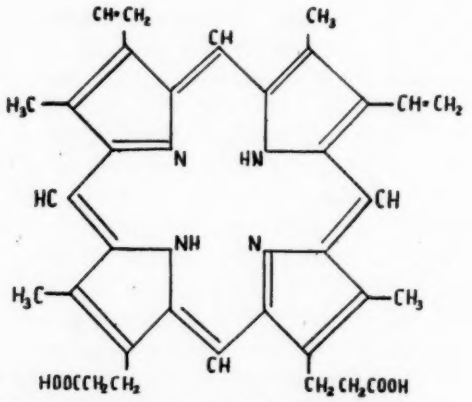

FIG. 5. Molecular structure of protoporphyrin.

It is appropriate here to mention that heme in combination with different proteins plays a role of outstanding importance in the life of both plants and animals. The compound of heme with globin known as hemoglobin is present in the red-blood cells of vertebrates and by its ability to combine reversibly with oxygen without undergoing oxidation performs the unique and indispensable role of a carrier for the storage and transport of oxygen in the organism. Another heme-protein known as myoglobin is the respiratory pigment occurring in muscle cells of both vertebrates and invertebrates. The cytochromes are the most widely distributed of all the heme proteins and occur in the cells of nearly all aerobic organisms, both plant and animal. Catalase and the peroxidases are other heme proteins which function as enzymes. This brief statement indicates the ubiquitous nature and extraordinary versatility of the heme proteins in performing biological roles of importance.

We shall later discuss the spectroscopic behaviour of heme in detail. It will suffice here to mention that all the heme proteins exhibit an intense absorption of light in the green region of the spectrum. Hemoglobin, for example, exhibits even in very dilute solutions a powerful absorption in the region of wavelengths between 5000 and 6000 angstroms, its maximum appearing around 5550 angstroms. That this absorption is a characteristic property of the porphyrin groups of compounds when a divalent metal atom occupies the central position in the molecule is shown by the fact that a powerful absorption with its maximum located at or near the same wavelength is also exhibited by several different porphyrins in combination with diverse metallic atoms which are divalent.

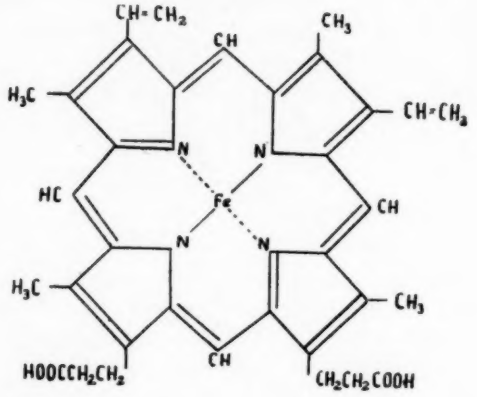

FIG. 6. The molecular structure of heme.

The considerations set forth above accordingly lead us to identify ferroheme as our pigment B. We thereby add to the list of the many biological functions performed by ferroheme that of being the principal mediator of vision in the photopic range. Indeed, the absorption of light by ferroheme is so strong that no great quantity of this pigment would be needed in the retina to enable it to function effectively as the mediator of vision. That it is indeed the principal visual pigment finds strong support in the fact that the position of its absorption maximum is not very different from the wavelength of maximum visual sensitivity in the spectrum. The generally accepted value for the latter is 5550 angstroms, but it may actually be a little greater for persons with normal colour vision.

That the pigment A which functions in the region of wavelengths between 4000 and 5000 angstroms is a carotenoid is indicated by the fact

that the absorption spectra of carotenoids appear just in that region. As already mentioned earlier, the two carotenoids that are invariably found in the green leaves of growing plants are β-carotene and xanthophyll. These colouring matters are also present in the plasma of human blood and in human milk in varying amounts, evidently as the result of the consumption of articles of food containing them. There is however a noteworthy difference between the two pigments, *viz.*, β-carotene is a precursor of vitamin-A, whereas xanthophyll is not. In other words, xanthophyll does not undergo fission and become transformed to vitamin-A in the human body. We may, therefore, with confidence assume that it is xanthophyll, and not β-carotene which is the pigment that finds its way into the human retina to function as a receptor in photopic vision.

The identification of pigment C has next to be considered. We have already noticed that while the absorption spectrum of this pigment appears principally in the yellow, orange and red regions of the spectrum, it also overlaps to some extent the region in which pigment B is operative, namely, the green of the spectrum. The reasons which make it probable that pigment C belongs to the same group of pyrrole compounds as pigment B have already been indicated. That the two pigments co-operate with other in producing the luminous effects described earlier on these pages is also significant. We are thus led to recognize that pigment C is closely related to pigment B. The suggestion that it is only an oxidised form of that pigment, in other words that it is ferriheme naturally presents itself. The spectroscopic and other evidence which supports and confirms this identification will be presented in a subsequent part of the present memoir.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part III. The Carotenoid Pigment

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(*Memoir No. 125 from the Raman Research Institute, Bangalore-6*)

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1. Introduction

In the first part of this memoir, the facts of observation regarding the luminosity and colour perceived at different points in the spectrum were set out in detail. The mechanism of perception and the role played by the visual pigments in the retina which would account for the facts were then discussed. In the second part of the memoir, the number of visual pigments which function and the spectral regions in which they are effective were deduced from certain luminous effects which were described, and the considerations which enable these visual pigments to be identified were also set out.

We shall now proceed a step further and show how it is possible to connect the observed variations of luminosity and colour in the spectrum with the absorption characteristics of the visual pigments which enable us to perceive light and colour. We shall in the first instance consider the blue-violet region of the spectrum which exhibits several features of interest, including especially the fact that in that region—and no other—the unaided eye is capable of recognizing polarised light and even of determining its plane of vibration by sight. It will be shown that these and other features are explicable in terms of the structure, spectroscopic behaviour and disposition in the retina of the molecules of the carotenoid pigment xanthophyll which was identified in the second part of the memoir as the mediator of photopic vision in that part of the spectrum.

2. The Carotenoid Pigments

The carotenoids are so named by reason of their chemical relationship to carotene which is a plant pigment first isolated in crystal form from the roots of the cultivated carrot. They are a numerous family of compounds and are essentially plant products. Indeed, there is no evidence

which would suggest that any carotenoid is produced *de novo* within the body of an animal. They enter the body by way of the articles of food which are consumed by the animal and if assimilated, pass into the blood stream to be utilised or stored up where needed. The potential vitamin-A activity is an important function of some of them, including especially β-carotene. This is however not one of the potential uses of xanthophyll since it is not a precursor of vitamin-A. Accordingly, xanthophyll is either accumulated in certain organs or else is passed out of the body.

An interesting example of the storage of xanthophyll is furnished by the yolk of the domestic hen's egg which, as is well known, exhibits a bright yellow colour. It is a convenient source-material from which xanthophyll can be obtained for the study of its spectroscopic behaviour. Repeated treatment with warm acetone results in the extraction of the pigment and on filtration, a clear liquid exhibiting a golden-yellow colour is obtained. This contains xanthophyll and also its isomer zeaxanthin as a minor constituent. As the latter has a very similar spectroscopic behaviour, its presence is not inconvenient for the purpose in view.

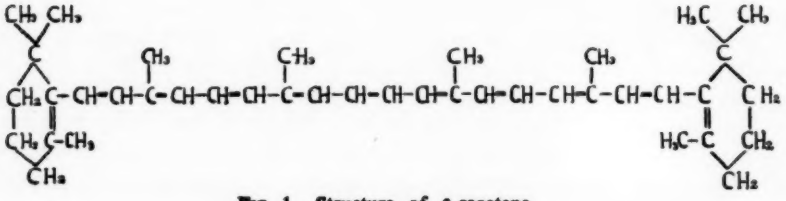

FIG. 1. Structure of β-carotene.

Carotene is a hydrocarbon having the chemical formula $C_{40}H_{56}$ which appears in different isomeric forms, the most important of them being β-carotene which has the structure shown in Fig. 1. It will be seen that the two end-groups in the molecule have an identical structure and are joined together by a long chain in which single and double bonds alternate. It is this system of conjugated double bonds that is responsible for the absorption of light by the substance which extends into the visible region of the spectrum. The characteristics of the absorption spectrum of β-carotene in hexane solution are exhibited in the spectrophotometer curve reproduced as Fig. 2. It will be seen that it is negligible at wavelengths greater than 5200 angstroms and that it rises very steeply in the region of wavelengths smaller than 5000 angstroms. After passing through a succession of maxima, it drops down but much less rapidly to quite small values at the violet end of the spectrum. The absorption maxima are located at 4770,

4500 and 4250 angstroms, the last being visible only as a point of inflexion on the curve.

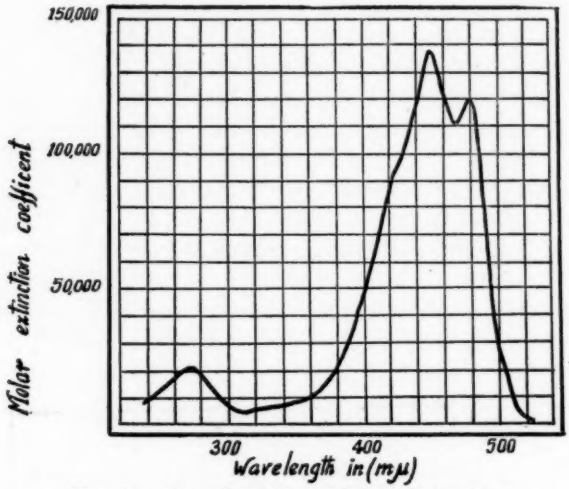

FIG. 2. Absorption characteristics of β-carotene.

Xanthophyll (also referred to in the chemical literature as lutein) has the formula $C_{40}H_{56}O_2$ and its structure which is shown in Fig. 3 exhibits its chemical nature as dihydroxy-α-carotene. The two end-groups in xanthophyll differ in their structure and this difference reflects the difference between α-carotene and β-carotene; the latter is symmetrical whereas the former is not. The characteristics of the absorption spectrum of xanthophyll in ethanol solution are exhibited by the spectrophotometer curve reproduced in Fig. 4. It will be seen that xanthophyll resembles β-carotene in its spectroscopic behaviour; but there are significant differences. In particular, the inflexion which appears in the absorption curve of β-carotene shows up as a distinct maximum at the same point in the case of xanthophyll.

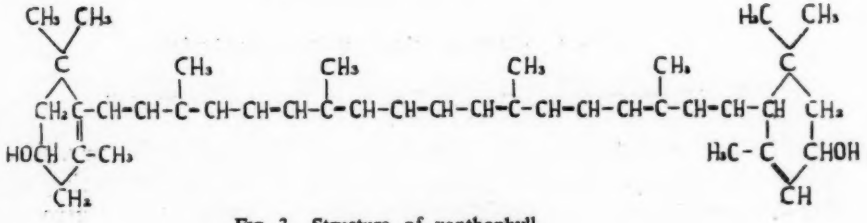

FIG. 3. Structure of xanthophyll.

The absorption spectra of the carotenoid pigments have been explained as arising from a swinging to and fro of the electrons along the length of their molecules. The incident radiation would excite such an oscillation provided that its electric vector is parallel to the length of the molecule. Simultaneously with the electronic oscillation and as an accompaniment to it, molecular vibrations would also be excited. The energy required for exciting the latter would add up to that of the electronic excitation. Hence the absorption spectrum would spread towards higher frequencies and smaller wavelengths. Peaks of absorption representing the successive harmonics of the most strongly excited molecular vibration frequencies may be expected to appear. It is thus possible to understand, at least in a qualitative fashion the characteristic features of the absorption curve: firstly, a steep rise at the long-wave end leading up to the first and fairly high maximum; after the first maximum, a few additional maxima and then a slow drop-down to a very weak absorption at the violet end of the spectrum.

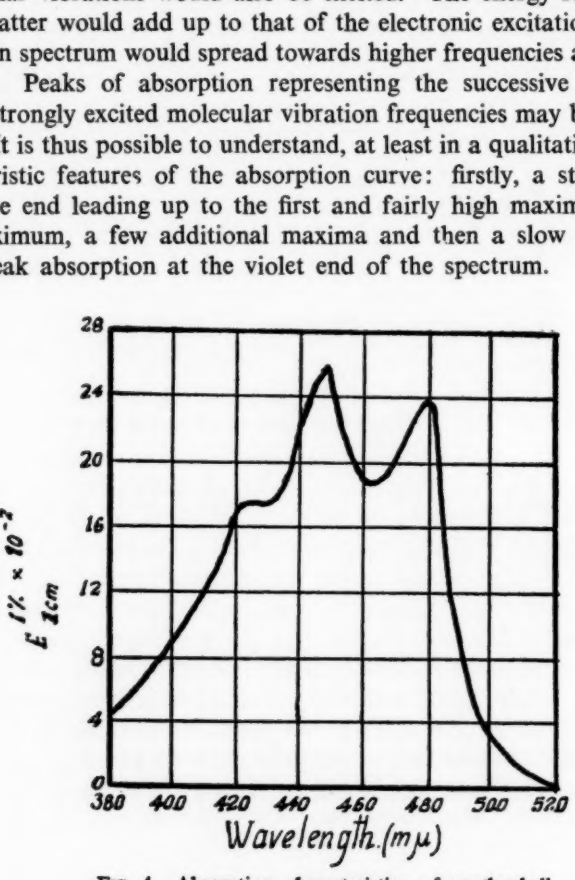

FIG. 4. Absorption characteristics of xanthophyll.

3. XANTHOPHYLL AND THE SPECTRAL COLOUR SEQUENCE

The colour of the spectrum to any observer with normal colour vision is green at 520 mμ, blue at 480 mμ and violet at 420 mμ. Referring to Fig. 4, it will be seen that the absorptive power of xanthophyll is zero at

520 mμ, has reached its first maximum at 480 mμ and that at 420 mμ it has passed its third maximum and is on its downward course. If xanthophyll is the visual pigment which functions in the wavelength range between 4000 and 5000 angstroms, the change-over from green to blue should appear in the vicinity of the steeply rising part of the absorption curve at about 490 mμ. This, indeed, is what is actually observed; the change of colour with wavelength is shown by the investigations already reviewed in the first part of this memoir to be very rapid in the vicinity of that wavelength. Similarly, one would expect the change from blue to violet to occur in the vicinity of the steeply falling part of the absorption curve around 440 mμ. At this wavelength, again, it has been observed that the colour changes rapidly with alteration of wavelength. In other words, the absorption-curve of the visual pigment and the colour sequence in the spectrum exhibit a close correlation, as indeed is to be expected since it is the visual pigment which by its absorptive properties enables the radiation to be perceived.

Fig. 5 reproduces the hue discrimination curve in the spectrum which appears on page 229 in the late Dr. P. J. Bouma's posthumously published book entitled *Physical Aspects of Colour*. It is stated that the curve represents the average of the results of a dozen different investigators whose published papers are listed in the book. (Some of these references are not available for consultation at Bangalore.) The graph shows a small dip at 420 mμ besides the more conspicuous ones at 490 mμ and 440 mμ, mentioned above. The dip at 420 mμ appears in the same position as the steep fall in the absorption curve of xanthophyll which commences after it has reached and passed its third maximum.

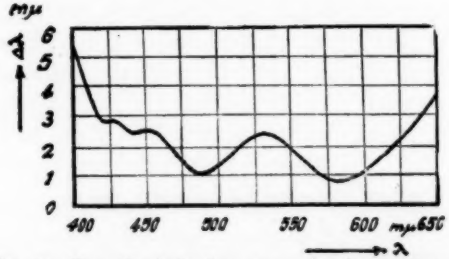

FIG. 5. Hue discrimination curve in the spectrum.

Besides the features referred to above, the blue-violet region of the spectrum exhibits other observable characteristics which should be mentioned and commented on here. The luminous efficiency which at 500 mμ is very much less than the maximum appearing in the greenish-yellow part of the spectrum shows a further rapid fall as we proceed towards shorter

wavelengths. This feature is represented in Fig. 6, the observations being those made by Jainski using a flicker method and a retinal illumination high enough to ensure that the results refer to photopic conditions. His observations do not take us beyond 460 mμ. But the simplest visual observations suffice to show that the luminosity of the spectrum becomes very small as we approach further towards its violet end. The other characteristic of the spectrum to which attention may be drawn appears in Fig. 5 reproduced above. The minimum of wavelength difference perceptible as a colour change exhibits a progressive increase as we approach the violet end of the spectrum, being some five times greater at 400 mμ than it is at 500 mμ. This increase accompanies and is superposed upon the undulations in the value of the minimum which appear in the same range of the spectrum. These features of visual experience are clearly related to the absorption characteristics of xanthophyll; the fall of luminous efficiency goes hand in hand with the decrease in absorptive power and the decreasing power of hue discrimination with the diminishing slope of the absorption curve, as we pass from the blue to the violet and approach the end of the visible spectrum.

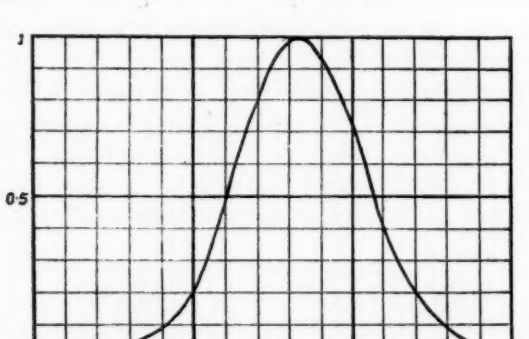

FIG. 6. Photopic luminous efficiency curve.

It appears worthwhile also to mention here that very simple visual observations with a pocket spectroscope and a moderately strong solution of xanthophyll prepared in the manner already stated suffice to establish the principal features in its absorption spectrum. With an absorption cell of small thickness, say 1 cm., one observes an enfeebled transmission in the blue and violet and a succession of absorption maxima in that region. This region exhibits a distinct edge at about 490 mμ beyond which there is

free transmission of light. A bluish-green section is visible which precedes the green and the rest of the spectrum. With a greater absorption path, say 3 cm., there is a complete cut-off of the violet and blue regions of the spectrum and a distinct enfeeblement of the bluish-green region. With a large absorption path, say 10 cm., the bluish-green section is also completely cut off, and we observe an absorption edge located at 510 mμ which separates the fully absorbed from the freely transmitted parts of the spectrum.

4. Effects Observed with Polarised Light

We have already had occasion to notice two very striking properties of xanthophyll, *viz.*, the elongated form of its molecules and the restriction placed on their power to absorb light and therefore also on the power of xanthophyll to function as a visual pigment, *viz.*, that the radiation falling on the molecules should have its electric vibration—or at least a component of it—parallel to the length of the molecules. These properties would have no observable consequences if the molecules of xanthophyll were orientated at random in the retina. This, however, is not the case in the foveal region which exhibits a depression or pit with the foveola at its centre and with sloping sides having a maximum slope angle of about 20°. As a consequence, the nerve fibres in the retina are normal to its surface only at the centre of the pit or depression. Elsewhere they slope away from the normal and as a result, the region of the fovea presents a radially disposed fibrous structure. The xanthophyll molecules are themselves highly elongated bodies and as they are part of the retina, they would naturally set themselves parallel to the elements of the fibrous structure in which they are located. In other words, the visual pigment would present to the incident light a radially disposed array of molecules with their long axes pointing towards the centre of the fovea. This radial disposition would be least evident at the centre of the fovea, would be most conspicuous in the region around the centre where the nerve fibres slope outwards and would cease again to be noticeable outside the foveal region.

If the observer views an extended source of unpolarised light, the special features of the foveal region referred to would not result in anything noticeable. With polarised light, however, the situation would be different. The molecules of xanthophyll which are parallel to the direction of optical vibration would function as absorbers and give rise to the sensation of a bright brush of light along that direction. The molecules perpendicular to the direction of vibration would be unable to absorb the light and hence a dark brush would appear along that direction. In other words, the observer would see an image of his fovea projected in space which presents the aspect

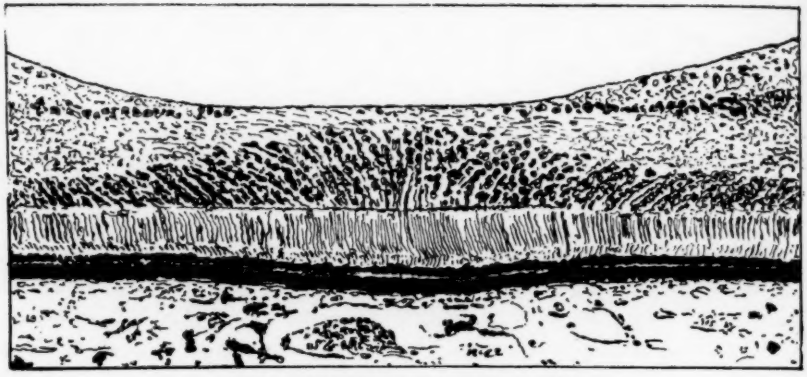

FIG. 7. Structure of the foveal region.

of a cross, the arm parallel to the direction of vibration in the incident light being bright and the arm perpendicular to it being dark.

Since the absorption of xanthophyll covers only the violet and blue regions of the spectrum but does not extend to greater wavelengths, it follows that the foveal cross seen in polarised light would be at its best if the incident light lies within those spectral regions. Light of greater wavelengths would not exhibit the phenomenon and hence its presence would only add to the general illumination of the field and thereby make the foveal cross a much less conspicuous phenomenon than it really is. This remark is of special importance in view of the relatively low luminosity of the blue and violet regions in comparison with the rest of the spectrum.

Since xanthophyll is a photopic visual pigment, it follows that the foveal cross requires for its observation that the illumination of the field with polarised light is sufficiently strong to ensure photopic conditions for the spectral region under consideration. When the illumination is reduced and we pass over to scotopic conditions, the effect should weaken and ultimately cease to be observable.

It should also be remarked that the phenomenon of the foveal cross should be equally well or even better seen binocularly as monocularly. For, what is actually observed is a picture of the foveal region projected on the field of view and when both eyes are used, their foveal regions are in register, in other words, both are seen at the same point in space.

It also deserves to be emphasised that the foveal cross seen in polarised light and the picture of the fovea seen with colour filters of various sorts

described in the second part of this memoir are closely related phenomena. In both cases we are concerned with a localised excitation of the visual receptors and the possibility of directly perceiving the results of such excitation. The closeness of the analogy will become plainer when we presently take up a description of the methods of observation and the results obtained.

5. Observation of the Effects

The phenomenon originally discovered by Haidinger, *viz.*, a faint brush which enables the eye to recognize polarised light and ascertain its plane of vibration is ordinarily both inconspicuous and fugitive in character. It however becomes a striking and conspicuous effect in the following circumstances: the field viewed by the observer should be of adequate intensity, *e.g.*, a cumulus cloud lit by sunlight or a brilliantly illuminated white screen; the observer should hold before his eyes (in addition to a polaroid) a colour filter which is transparent to the blue and violet rays and completely cuts out the rest of the spectrum; the polaroid should be rotated, or else oscillated continuously instead of remaining in a fixed orientation. With these arrangements, one observes a dark brush and crossing it transversely, a bright brush; the dark brush is very dark and the bright brush is brighter than the surrounding field. The two brushes together form the figure of a cross which fills an area which is readily recognizable as a projection in space of the fovea centralis of the observer's own retina. As the polariser is turned round or oscillated, the cross turns round or oscillates synchronously with it around a point which is the projection in space of the foveola of the observer's retina. If the polaroid and colour filter are both large enough, the whole phenomenon can be viewed binocularly.

The importance of using a colour filter which transmits the blue and violet regions of the spectrum and excludes the rest becomes clear when the observer substitutes for it a complementary filter, *viz.*, a yellow glass plate which cuts off the blue and violet regions and transmits freely the rest of the spectrum. *Not a trace of the foveal cross can then be seen.* It follows that the cross owes its origin to a visual pigment whose absorption appears in the violet and blue regions but does not extend to the rest of the spectrum. A more detailed examination may be made in different ways. One can, for example, view through the polaroid a field illuminated by a monochromator and alter the wavelength progressively. Alternatively, we may use the monochromatic radiations of the mercury arc; the brushes are fairly well seen with λ 4538 and also, but not so well, with the λ 4046 radiations. A simple method by which the entire spectrum may be scanned is to view the first order spectrum of a linear source of white light produced by a glass

diffraction grating held along with a polaroid in front of the observer's eye. The foveal cross can then be seen, if the observer directs his vision to the blue or violet region of the spectrum. But they are not visible in other parts of the spectrum, the transition from invisibility to visibility occurring rather abruptly around 4900 angstroms. The cross is clearest in the wavelength region between 4800 and 4300 angstroms. It is less distinct at still shorter wavelengths but nevertheless continues to be visible to the violet end of the spectrum.

A technique of observation different from that described above yields highly interesting results. With the colour filter held before his eye, the observer suddenly interposes a polariser in front of it. The cross then comes into view and slowly fades away. After a little while, the observer suddenly removes the polaroid. The cross is then seen once again but rotated through a right angle, the dark arm appearing in the place of the bright arm and *vice-versa*. On putting the polariser back, the cross regains its original configuration; and when the polariser is taken out once again, the cross turns round once again. It is thus clear that a sudden removal of the polaroid produces an effect of the same nature as that of turning it round through a right angle. This effect is clearly analogous to the phenomena observed with colour filters described in the second part of this memoir. The sudden removal of the polaroid results in the component vibration cut off by it being restored and allowed to fall upon the retina. As a consequence, the foveal cross is seen again but turned round through a right angle.

A convenient technique for studying the effects due to polarised light at different levels of illumination is to use a powerful and completely enclosed source of light, *e.g.*, a mercury arc or a tungsten lamp and to isolate the effective part of its spectral radiation by a colour filter which covers an aperture placed close to the source. The light which issues from the aperture can be received on a translucent diffusing screen and the light emerging through the latter is viewed by the observer through a polaroid from an appropriate distance. If the arrangement is set up in a long darkened chamber, the brightness of the field under observation can be varied over a large range by the simple device of moving the diffusing screen from a position close to the aperture from which the light issues to another sufficiently far away. Observations made in this manner show that the foveal cross is visible only when the illumination which falls on the diffusing screen is strong enough to allow ordinary print to be read. If the illumination be diminished further so that print ceases to be readable, the cross becomes

indistinct. When the illumination is so feeble that a printed page appears to the eye as a mere blur, the cross ceases altogether to be visible. The disappearance of the foveal cross thus goes hand in hand with the disappearance of the visual acuity which is a characteristic of photopic vision. It is also observed that the field of view as seen through the polaroid shows a progressive change in colour from a brilliant blue to a pale blue as the illumination is diminished to the point at which the foveal cross ceases to be noticeable. This is a further indication that we have then moved out from the photopic to the scotopic level of illumination in the blue and violet regions of the spectrum.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part IV. Ferroheme and Ferriheme

By Sir C. V. Raman

(*Memoir No. 125 from the Raman Research Institute, Bangalore-6*)

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1. Introduction

In the first part of this memoir, the basic facts concerning the perception of light and colour were reviewed and a mechanism of the functioning of the retina was suggested which explains them in a simple and intelligible fashion. Human vision is mediated by certain pigments present in the retina, these pigments acting as energy-receiving and energy-transferring agents; in other words, they absorb the quanta of radiational energy incident on the retina, but pass on the energy thus absorbed to the sensory mechanism, themselves returning to their original states. The second part of the memoir dealt with the problem of determining the number and nature of the visual pigments functioning in the manner indicated, the regions of the spectrum in which they respectively operate and the distribution of the pigments over the area of the retina. A method of observation was described which furnishes valuable information on these points. Considerations were also developed which pointed to xanthophyll, ferroheme and ferriheme as the three visual pigments with which we are concerned in photopic vision. In the third part of the memoir, evidence was presented which confirms and establishes that xanthophyll is the visual pigment which functions in the violet and blue regions of the spectrum.

In the present part of the memoir, we are concerned with the region of the spectrum between the wavelengths 5000 and 7000 angstroms. The facts of observation which concern us here and need interpretation are firstly, the distribution of luminosity in this region of the spectrum; secondly, the distribution of colour in it; and thirdly, a derivative property of the same, namely, the characteristics of the colour progression which find expression in the so-called hue discrimination curves determined by various observers. We shall consider these facts here in some detail and discuss their interpretation. The aim is to infer therefrom the spectroscopic behaviour

of the visual pigments and to compare the same with what is known regarding the absorption spectra of the heme pigments.

2. Luminosity, Colour and Hue Discrimination

The form of the luminous efficiency curve in the spectrum depends to a notable extent on the region of the retina used in its determination. In what follows, we shall make use of the data obtained by Walters and Wright under photopic conditions in which only the foveal region of the retina was employed. Their results are reproduced below in Fig. 1. It will be noticed that the luminous efficiency exhibits a well-defined maximum at 5600 angstroms on either side of which it descends steeply, but less rapidly so on the side of longer wavelengths than towards the shorter ones. This pronounced asymmetry of form evidently calls for explanation.

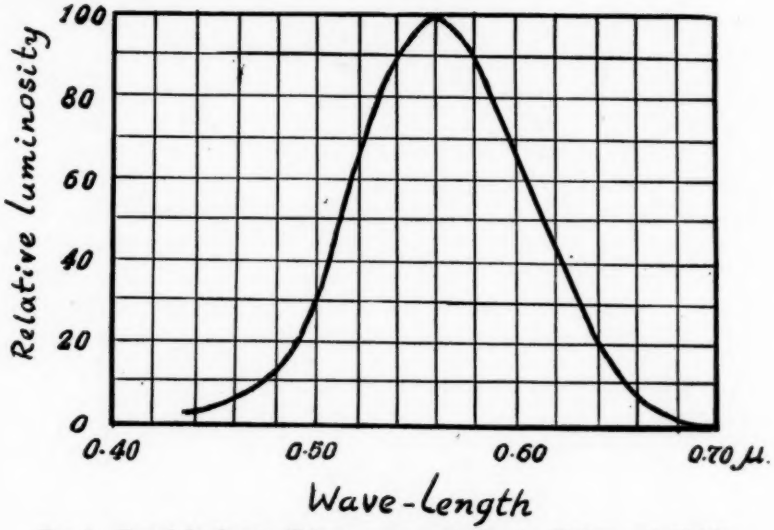

Fig. 1. Foveal luminous efficiency curve according to Walters and Wright.

The graph of the luminous efficiency reproduced in Fig. 1 may be divided into four parts which exhibit distinctly different characters: (i) the foot of the graph from 4400 to 4950 angstroms where it exhibits a marked curvature; (ii) the steeply ascending part from 4950 to 5600 angstroms; (iii) the steeply descending part from 5600 to 6270 angstroms; (iv) the foot of the graph from 6270 to 7000 angstroms where again it exhibits a marked curvature.

On the basis of the average positions of the colour boundaries in the spectrum as placed by observers with normal vision, we may divide the

visible spectrum into four sectors thus: Blue-violet, $\lambda < 495\ m\mu$; Green. $495\ m\mu < \lambda < 566\ m\mu$; Orange-yellow, $566\ m\mu < \lambda < 627\ m\mu$; and Red, $627\ m\mu < \lambda$. It will be noticed that these sectors represent also the divisions of the luminous efficiency curve indicated above, except that the green-yellow boundary is placed at 566 mμ instead of 560 mμ where the maximum luminous efficiency appears.

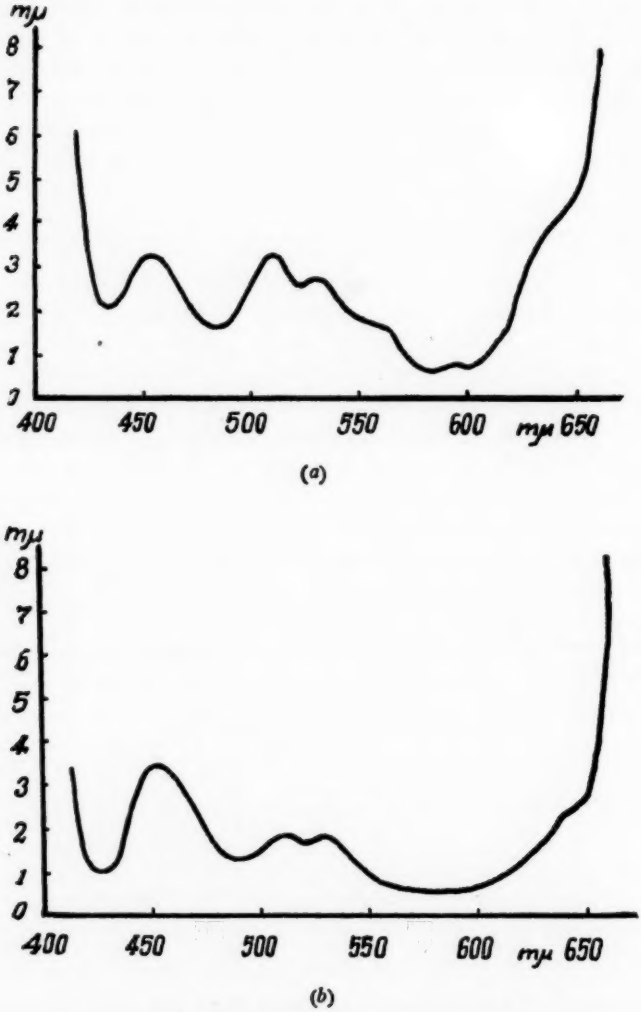

FIG. 2. Hue discrimination in the spectrum (after Haase).

Measurements of the smallest change of wavelength needed for a perceptible change of colour have been made and reported by numerous observers. Their results agree in respect of the major features but show some differences in detail. This is not surprising, since the characteristics of human vision are by no means the same for all observers, and the techniques and physical conditions of observation are also not identical in all the investigations. Two slightly different forms of these hue discrimination curves have already been reproduced in earlier parts of this memoir. Figure 2 (*a*) and Fig. 2 (*b*) above represent the results reported by Haase for two different levels of illumination, Fig. 2 (*b*) representing the results for the higher level of the two which was ten times greater in intensity than the other. Figure 3 below reproduces the results of E.P.T. Tyndall for the wavelength range between 450 mμ and 650 mμ. The individual observations made by him at various times are marked in the graph by crosses and circles and show a remarkable consistency except in the vicinity of the humps at 530 mμ and 630 mμ respectively.

Tyndall's observations place the wavelength of minimum limen at 575 mμ where it has the value of 0·5 mμ; at greater wavelengths it increases, at first quite slowly and then more and more rapidly, reaching large values at the red end of the spectrum. The results reported by Haase and shown in Figs. 2 (*a*) and 2 (*b*) exhibit generally similar features in the same region. At the wavelengths around 530 mμ where Tyndall's data exhibit a considerable scatter, Haase's results show two minor humps instead of the single hump at 535 mμ reported by others.

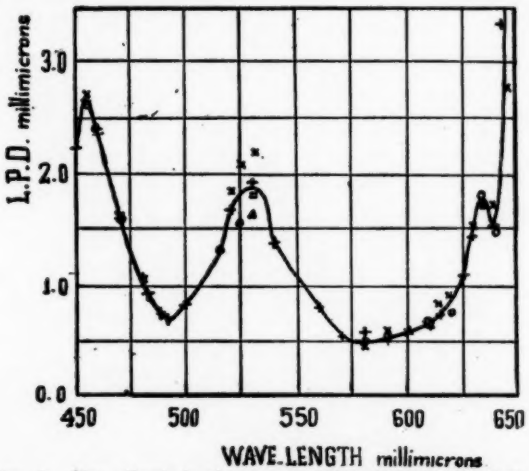

FIG. 3. Hue discrimination curve (after E. P. T. Tyndall).

3. THE COLOUR SEQUENCE IN THE SPECTRUM

The luminous efficiency curve reproduced in Fig. 1 above represents a summation of the effects of the three visual pigments functioning in their respective regions of the spectrum. It gives no indication of any special features related to those appearing in the hue discrimination curve at various points in the spectrum. We have therefore to assume that any special features appearing in the absorption curves of the individual pigments which determine their respective luminous efficiencies have been smoothed out and rendered unobservable by reason of such summation. However, since the econd visual pigment which functions in the green sector of the spectrum plays the major role in human vision, we are justified in assuming that the pronounced maximum at 560 mμ exhibited by the luminous efficiency curve arises principally by reason of a very conspicuous maximum at or near the same wavelength in the absorption spectrum of that pigment. On the other hand, in the blue-violet region of the spectrum, the first visual pigment which functions in that region would principally be responsible for the observed luminous efficiency. Likewise, the third visual pigment would be responsible for the observed luminosity in the region of longer wavelengths and its extension in that direction would account for the asymmetric form of the luminous efficiency curve.

The experiments and observations described in the second part of this memoir showed very clearly that there is a considerable overlap in the absorption spectra of the second and third visual pigments. That there is such an overlap and that it has a most important effect on the visual sensations excited by light appearing in the regions of such overlap is the clue to an understanding of the facts set forth above regarding the distribution of colour in the region of the spectrum now under consideration. The luminous efficiency curve itself indicates that there is a large drop in the absorptive power of the second visual pigment at wavelengths greater than 560 mμ and we may safely assume that this drop continues over the whole range in which that curve goes steeply down, in other words, up to about 627 mμ. The colour change from green to yellow, from yellow to orange and then from orange to red appears precisely in this region. It is a justifiable inference that these changes are a consequence of a rapid falling off in the luminous effect due to the second visual pigment and its progressive replacement by the luminous effect of the third visual pigment which, though inherently much weaker than that of the second pigment, nevertheless effectively determines the observed visual sensations in the regions of the spectrum where it is relatively more important. Continuing this line of

argument, we infer that when, at about 627 mμ, the colour of the spectrum passes over from orange to red, the second visual pigment has ceased to be effective, in other words, that its absorption strength is practically zero. On the other hand, the luminous efficiency curve itself indicates that the visual effect due to the third pigment persists up to the extreme red end of the spectrum, slowly and progressively decreasing with increasing wavelength.

4. Hue Discrimination in the Spectrum

The mechanism of the perception of light and colour by the eye which was indicated in the first part of the memoir was based on the quantum theory of radiation. It recognizes that the physical basis for the differences in colour perceived by the eye in the different parts of the spectrum is the fact that the magnitudes of the light-quanta differ from one end of the visible spectrum to the other, being least at the red end and greatest at the violet. To account for the remarkably high power of discrimination of colour actually exhibited by our eyes, it was postulated that the visual pigments in the retina which are the mediators of vision absorb the energy quanta incident on them and immediately pass on the energy thus absorbed to the sensory mechanism and return to their original energy states. Accepting this hypothesis, the following two questions arise which require an answer: What is the factor which limits the power of the eye to discriminate differences in colour in different parts of the spectrum? Why is it as great as it is in some parts, and why is it less in others? We shall now endeavour to find answers to these questions.

As is well known, the absorption of light by molecules embedded in solid or liquid media is manifested in the form of diffuse spectral bands exhibiting only remnants of the structure shown by their spectra in the state of vapour. As a typical example, we may mention the absorption spectrum of toluene in hexane solution observed in the near ultra-violet. Some nineteen bands are indeed discernible in the region between 270 mμ and 240 mμ, but only the first few of them are sharp and intense, and as we proceed further into the ultra-violet, they become weaker and more diffuse, and the individual bands can only with difficulty be distinguished apart from each other.

The absorption of light by the molecules of the visual pigments embedded in the retina and the transfer of the absorbed energy to the sensory mechanism would necessarily be influenced by various factors, including especially the thermal agitation in the medium. The absorption of radiation involves the electronic energy levels of the molecules of the pigment and the vibrational levels coupled with them. Thermal agitation, on the other hand,

appears as the energy of translatory movements. In solid and liquid media, the molecules may be regarded as being continuously in a state of collision, and hence exchanges of translatory energy *may* occur simultaneously with the exchanges of electronic and vibrational energy. Whether this happens at all and the extent to which it occurs may be expected to depend on the circumstances of each particular case. To obtain a rough idea of the effect of such exchanges, we proceed on the basis of the highly simplified picture of the process indicated by the following equation

$$h\nu - h\nu^* \approx \pm kT$$

in which h, k, and T are respectively Planck's constant, Boltzmann's constant and the absolute temperature of the retina, while $h\nu$ is the energy of the light incident on it and duly absorbed and $h\nu^*$ is the energy actually transferred to the sensory mechanism. The plus and minus signs refer to the cases in which the energy transferred is respectively diminished and increased by the presence of thermal agitation.
Taking

$$h = 6{\cdot}62 \times 10^{-27} \text{ erg.sec.}$$
$$k = 1{\cdot}38 \times 10^{-16} \text{ erg.deg.}^{-1}$$
$$T = 310°$$

$(\nu - \nu^*)$ when expressed as wave-numbers comes out as ± 215. In other words, the precision with which the eye can recognize a variation in the magnitude of the light quantum as a variation in colour in the spectrum would be diminished to the extent of 430 wave-numbers. When expressed as a wavelength spread in millimicrons, it comes out as directly proportional to the square of the wave-length in the spectrum, in other words, some four times greater at the red end of the spectrum than at the violet end. The calculated figures are shown in Table I below.

TABLE I

Effect of thermal agitation on hue discrimination

Spectral region in mμ	750	700	650	600	550	500	450	400
Spectral spread in mμ	24	20	18	16	12	10	8	6

Comparing the figures shown in Table I with the actual facts of observation as reported by various investigators, it is immediately obvious that except at the extreme red and extreme violet ends of the spectrum, the eye actually exhibits a power to detect changes of colour with change of wavelength far in excess of that indicated by the calculations appearing in Table I. Especially in certain parts of the spectrum, *viz.*, around 440 mμ, 490 mμ and 590 mμ, one finds a remarkable and obviously highly significant sensitivity to colour change with wavelength which closely approaches the theoretical perfection indicated by the principles of the quantum theory when the effect of thermal agitation is ignored. The inference is obvious, *viz.*, that the manner of calculation adopted greatly overestimates the influence of thermal agitation on hue discrimination. The transference of radiational energy to the sensory mechanism which takes place at the retina through the medium of the absorbing pigments present in it is presumably a very rapid process, and it is by no means inevitable that it would be influenced by the thermal agitation in the medium in the manner and to the extent contemplated in the calculations.

We are thus compelled by the facts to approach the subject of the varying power of hue discrimination in the spectrum from a different standpoint. We may reasonably expect it to be closely related to specific features in the absorption spectra of the visual pigments functioning in the respective regions of the visible spectrum; these features are not necessarily observable in the luminous efficiency curve, being masked by reason of the superposition of the effects of all three pigments in it. In the hue discrimination curve, on the other hand, they may well be expected to manifest themselves. In other words, the form of the hue discrimination curve is an indicator of the features of the absorption spectra of the individual pigments and is thus of prime importance in relation to the physiology of vision.

Already, in the third part of this memoir, it has been shown that the form of the hue discrimination curve in the blue-violet sector of the spectrum stands in the closest relationship to the special features in the absorption spectrum of the visual pigment functioning in that sector, *viz.*, xanthophyll. The minima of the limen of wavelength change for a perceptible alteration in colour appearing around 490 mμ and 440 mμ were, in fact, explained as due to the steep ascent and descent appearing in the absorption spectrum of xanthophyll respectively at those wavelengths. It is evident that we have likewise to seek for the explanation of the very remarkable minimum of limen noticeable in the wavelength region around 590 mμ on a similar basis.

Earlier, it has been remarked that the drop of the luminous efficiency at wavelengths greater than that of its maximum at 560 mμ should be

ascribed to a steep drop in the absorptive power of the second visual pigment in that region. Indeed, the latter drop should evidently be even steeper than that of the luminous efficiency curve. For, in the latter curve, the effect of the third visual pigment is superposed and since the latter evidently diminishes with increasing wavelength in this region, the steepness in the drop of the luminous efficiency curve would be diminished by reason of such superposition. We are thus entirely justified in inferring that in the region of wavelengths greater than 560 mμ, the second visual pigment exhibits a very steep drop in absorption with the result that it is practically negligible at wavelengths greater than 625 mμ. Accordingly, in the region of wavelengths between 560 mμ and 625 mμ, we should expect the limen for colour discrimination to exhibit a large diminution and that it should reach its lowest value in the region of wavelengths somewhere between those limits where the absorption curve of the second visual pigment exhibits its steepest fall, *viz.*, around 590 mμ. This is what is actually observed to be the case. The two D lines of sodium appear in this vicinity, and it is a well-established experimental result that when these two lines are equalised in their intensity, they exhibit an observable difference in colour.

5. THE ABSORPTION SPECTRA OF THE HEME PIGMENTS

The considerations of a general nature which lead us to the identification of the two visual pigments functioning in the green and red sectors of the spectrum as ferroheme and ferriheme respectively have already been set out in the second part of this memoir. We shall, in what follows, review the known facts regarding the absorption of light by the heme pigments with a view to compare them with the behaviour of the retinal pigments as indicated by the facts of visual experience.

The iron-protoporphyrin complex known as heme appears associated with different proteins in biological material. The extraordinary versatility which heme exhibits in performing a variety of physiological functions is ascribable to its association with the appropriate proteins in the different circumstances. Extensive studies have appeared in the literature regarding the absorption spectra which it exhibits in various cases. Indeed, the identification of the different heme proteins in biochemical research is largely based on their spectroscopic behaviour. The absorption of light by the heme proteins owes its origin in the first instance to the special structure characteristic of the porphyrin group of compounds, *viz.*, the tetrapyrollic group containing a closed ring of eighteen bonds which are alternately single and double. The character of the absorption is however modified when a metal atom enters the porphyrin structure and finds its place at the centre

of the ring. Further modifications in the spectroscopic behaviour appear when the metal-porphyrin structure associates itself with other nitrogenous materials, including especially different proteins.

The porphyrins when dissolved in organic solvents exhibit a typical four-banded absorption in the visible spectrum with some indications of further details. The strength of the bands increases towards shorter wavelengths and in addition there is a still stronger band at about 400 mμ known as the Soret band, having been first observed by that author in the absorption spectrum of hemoglobin. The four bands of protoporphyrin, for example, in ether-acetic acid solution have their maxima at 632, 576, 537 and 502 mμ respectively, while indications of subsidiary bands at 605 and 585 mμ have also been noticed. It is worthy of remark that at the temperature of liquid air, the bands sharpen and are partly split up and shifted to shorter wavelengths.

The spectroscopic behaviour of the porphyrins in the form of complex salts formed by their combination with different metals has been extensively investigated, using the synthetically prepared substances in solution in different organic solvents. Generally speaking, it is found that the four-banded spectrum of the porphyrins is replaced by a two-banded spectrum, the position of the two maxima as well as their relative intensity varying with the metal which has entered the structure of the porphyrin. Ferrocoproporphyrin, for example, shows a strong absorption band at 550 mμ and a weak one at 520 mμ. The great difference between the spectroscopic behaviour of the complexes formed by the combination with iron in the ferrous and ferric states may be illustrated by the case of mesoporphyrin. Whereas the ferrous compound with it exhibits, in a buffered acetic acid solution, a band covering the region 555 mμ to 565 mμ in the green, the ferric compound shows two bands, one in the green between 530 mμ and 542 mμ and another in the red between 630 mμ and 640 mμ.

The association of the iron-porphyrin complexes with other nitrogenous substances to form what are known as hemochromes and hemichromes respectively has a notable influence on the character of their absorption spectra. The hemochrome structure is characterised by the appearance of a two-banded absorption spectrum of which the first or α-band is very sharp and also much more intense than the second or β-band of shorter wavelength. The positions of these two bands are observably influenced both by the nitrogenous base and by the porphyrin. In the hemochrome formed by protoporphyrin with pyridine for example, the α-band has its maximum at 558 mμ, while the β-band appears at 525 mμ.

On the other hand, the hemochrome formed by the combination of ferrous iron with mesoporphyrin and pyridine has its absorption maxima at 547 mμ and 518 mμ.

A reference should also be made here to the absorption spectra of the cytochromes, of which several have been reported and which are distinguished from each other by their spectroscopic behaviour. The best-known of them is cytochrome *c* which has been isolated and spectroscopically investigated in the pure state. It shows absorption maxima at 550 mμ and 521 mμ, the former or α-band being the more intense of the two. Figure 4 below reproduces the absorption characteristics of cytochrome *c* as determined by Theorell.

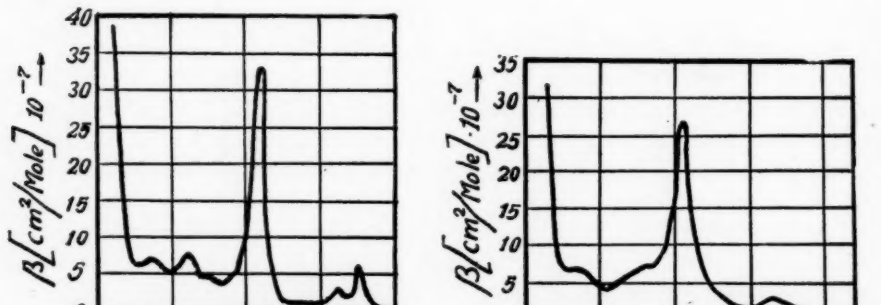

FIG. 4. (*a*) Ferrocytochrome. (*b*) Ferricytochrome. Absorption spectra of cytochrome *c* after (Theorell).

Figure 4 (*a*) represents that of the reduced form, *viz.*, ferrocytochrome *c* and Fig. 4 (*b*) that of the oxidised form, *viz.*, ferricytochrome *c*. It will be noticed that while the absorption by the ferrous pigment exhibits a strong, sharp band located at 550 mμ and a weaker one at 521 mμ and is practically confined to the green region of the spectrum, the ferric compound has an absorption extending into the red with a broad diffuse maximum in the green around 530 mμ. This difference between the ferrous and ferric forms is further illustrated in Fig. 5 (due to Horecker and Kornberg) which shows that the absorption by the latter goes right up to the extreme red end of the spectrum.

6. THE OBSERVABLE CONSEQUENCES

If white light is spread out into a spectrum of small dispersion by a diffraction grating, the latter appears to the eye to consist of three bands

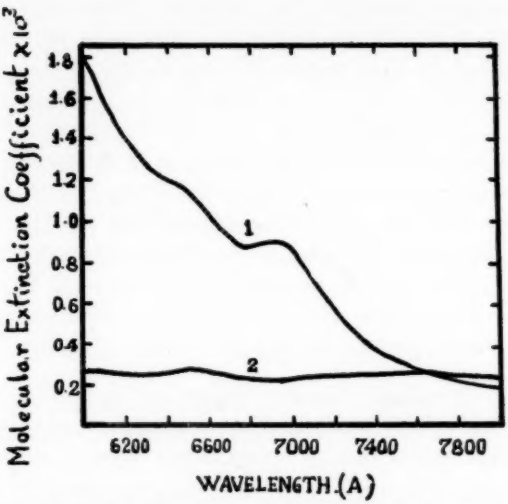

FIG. 5. Absorption spectrum of cytochrome c in the extreme red.
(1) Oxidised form; (2) Reduced form.

of colour of approximately equal width: a blue-violet band which is highly colourful but of low luminosity and covers the region from 400 mμ to 500 mμ; a green band from 500 mμ to 600 mμ and a red band from 600 mμ to 700 mμ. The two latter bands are also colourful, the red perhaps more so than the green, but the red is less luminous, especially near the extremity of the spectrum. The yellow-orange section of the spectrum appears merely as an edging between the green and the red. The most luminous part of the spectrum is in the green, not far from its yellow edge. The rapid changes in colour from blue to green at about 490 mμ, and from yellow to orange at about 590 mμ are also obvious to inspection. One is tempted to associate the three principal bands of colour with three visual pigments functioning in three distinct regions of the spectrum. But the appearance of the yellow and orange as a transition between green and red is a clear indication that there is an overlap of the absorption spectra of the second and third visual pigments.

The various related features of the spectrum mentioned above are readily intelligible in the light of the spectroscopic behaviour of the heme pigments discussed in the foregoing pages. The pigments formed by the combination of ferrous iron with protoporphyrin absorb light in the region of wavelengths between 500 mμ and 600 mμ. In all cases, the maximum of this absorption appears at or near 560 mμ which is also the wavelength

of maximum luminous efficiency in the spectrum. Then again, the absorption drops down quickly with increasing wave-length between 560 mμ and 600 mμ. This feature explains the low values for the limen of hue discrimination observed in that region. The absorption by the ferric forms of the pigment, on the other hand, appears both in the green and red sectors of the spectrum; it is, however, weaker and more diffuse than that of the ferrous forms. This situation explains the appearance of yellow and orange as transition colours between the green and the red in the region where the absorption by the second pigment is weak. Likewise, the diffuseness of the absorption by the ferric pigment and its progressive weakening with increasing wave-length explain the diminishing luminosity and the increasing limen of hue discrimination as we approach the red end of the spectrum.

Some remarks should be made here regarding the Soret band of absorption. Had this been effective as a mediator in human vision, the violet end instead of being the feeblest part of the spectrum, would have been the most luminous. The inference is obvious, that the Soret band is not active as a transmitter of the energy absorbed which, presumably, is dissipated in some other fashion.

The Soret band is a general feature in the absorption spectra of the whole porphyrin group of compounds, even when a metal atom is not present at the centre of the tetrapyrollic group. There is therefore no reason to believe that it would act in the same way as the absorption bands in the visible region appearing by reason of the presence of the metal atom. It may be remarked in this connection that chlorophyll participates in the photosynthetic activity of green leaves by reason of its characteristic absorption band in the red; the Soret band, so far as is known, remains entirely inactive.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part V. The Colour Triangle

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1. Introduction

So far, in this memoir, we have concerned ourselves exclusively with the sensations excited by monochromatic radiations of different wavelengths appearing in the spectrum. The reason for this, as has already been explained in the first part of the memoir, is that only by such an approach is it possible to reach a correct understanding of the nature of the retinal processes which enable us to perceive light and colour. We shall now turn to the consideration of the more complex field which offers itself in the study of the sensations excited by heterogeneous light. Here again, the visual sensations resulting from monochromatic light necessarily form the starting-point of our approach to the subject. Indeed, the outstanding result which has emerged from all investigations in this field is the relation that all observed colours bear to the colours of monochromatic radiation. These latter stand in a category by themselves and form a kind of upper limit to the visual manifestations of colour.

The functioning of the three visual pigments present in the retina in their respective spectral regions will form the basis of our considerations. It will be shown that they enable a satisfactory elucidation to be given of the observed facts of the subject including especially those which in the past have been sought to be interpreted or explained in terms of the so-called trichromatic theory of vision. It is necessary here to emphasise that for a full understanding of the facts of heterochromatic vision, the role played by the central parts of the organ of sight is no less important than the functioning of the retina which is only the periphery of that organ. The function of the retina is to receive, absorb and pass on the energy of the incident radiation. But the synthesis which enables composite radiation consisting of energy quanta of different magnitudes to be perceived as a visual sensation can only take place in the central part of the visual organ. This is indeed

very clear from the facts of binocular vision. There is no colour sensation which can be produced by mixing two lights and presenting them to one eye which cannot be duplicated by supplying the two lights independently, one to each eye. As an example of this general principle, it will suffice to mention the familiar techniques employed in colour stereoscopy.

2. THE CHROMATIC SENSATIONS

As we proceed, it will emerge that the sensations which result from the superposition of radiations appearing in different parts of the spectrum fall into two categories which we shall term the *chromatic* and *achromatic* sensations respectively. We shall commence with a consideration of the chromatic sensations. The colours of the spectrum which represent the effect of monochromatic radiations on our visual organs are, of course, the chromatic sensations of the first order. In certain circumstances, however, the superposition of different monochromatic radiations may result in colour sensations which may be included in that category. We shall now consider these cases in order.

A group of cases of particular importance is that in which two radiations appearing respectively at the two ends of the spectrum, *viz.*, violet and red, are superposed. The observations described in the second part of this memoir show that the visual pigments which function at the two ends of the spectrum are *exclusively* the first and the third respectively. The energy-quanta at the red and violet ends of the spectrum also differ widely. There is no reason, therefore, to anticipate that the spectral components of the incident radiation would be confused with each other when the signals originating at the retina reach the cerebrum. Indeed, in this case, human vision very nearly succeeds in recognising the composite nature of the incident radiation. That the so-called purples are a mixture of red and violet is fairly obvious even to an inexperienced observer. The relative intensities of the two components make themselves felt in the hues perceived which form a complete sequence ranging from red at one end to violet at the other and rival the pure colours of the spectrum in their brilliance. It follows that the purples can be classed with the colours of monochromatic light as chromatic sensations of the first order. It is evident also that a mixture of two purples in any proportion would give us only another purple, in other words, nothing essentially different.

Another set of cases of special importance is that in which the two monochromatic radiations which are superposed both lie within the range of wavelengths between 530 mμ and 780 mμ. Xanthophyll which is the visual pigment functioning in the violet and blue sectors of the spectrum

does not absorb any light of wavelengths greater than 530 mμ. Hence, in the region between 530 mμ and 780 mμ, only two visual pigments, *viz.*, ferroheme and ferriheme function. The observations described in the second part of the memoir show clearly that there is a considerable overlap in their absorption spectra. It follows that when red and green radiations from the two ends of the range are superposed in any proportion, the resultant sensation would be one of the spectral colours falling within the same range. Indeed, two monochromatic radiations from anywhere between these wavelengths when superposed would reproduce a spectral colour lying elsewhere in the same range. These indeed are facts. They emerged quite clearly from Clerk Maxwell's investigations with his colour box and have been confirmed by all later investigations.

There is yet a third class of cases in which the superposition of monochromatic radiations gives rise to a chromatic sensation, *viz.*, those in which the superposed radiations are close to each other anywhere in the spectrum. They may be sufficiently far apart to be perceived as different in colour when viewed separately or in adjacent fields. Yet, when they are superposed, the eye fails to recognise the composite nature of the light and perceives a colour which may be described as the colour of a spectral frequency which is the weighted average of the frequencies of the superposed radiations, the weightage being determined by their respective luminosities.

3. The Achromatic Sensation

A spectroscopist would define white light as a stream of radiation which comprises energy-quanta of all possible values ranging over the entire visible spectrum and with an energy distribution such as would be found in the radiation from a black body at very high temperatures. Since, however, the central organ of vision is incapable of resolving the incident radiation into its spectral components, there is no reason for assuming that only such a radiation would be perceived by the eye as white light. Indeed, much less stringent requirements might suffice. We may remark here that the light falling on the retina is absorbed by three visual pigments which between them cover the entire range of the visible spectrum. Hence, the minimum requirement for the perception of the incident heterogeneous radiation as white light could well be the following: *all the three visual pigments should function and should contribute to the observed luminosity in the same proportions as they would if the incident radiation were white light in the spectroscopic sense.* We shall provisionally accept this requirement as adequate and compare its consequences with the actual facts of observation.

The green light appearing in the wavelength region between 495 mμ and 566 mμ stands in a category by itself. In this sector of the spectrum, vision is mediated almost exclusively by ferroheme though the other two visual pigments make sensible contributions respectively near the two ends of the sector. It follows that to achromatise green light, one would require the addition of radiations from both ends of the spectrum where xanthophyll and ferriheme respectively function. The complementaries to the green of the spectrum accordingly lie in the region of the purples; as we pass from the boundary between blue and green to the boundary between green and yellow, the location of the complementary colour would shift from the red to the violet end of the series of purples.

As has been remarked earlier, the yellow colour of the spectrum between 566 mμ and 589 mμ and the orange colour between 589 mμ and 627 mμ arise by reason of the circumstance that the absorption spectra of ferroheme and ferriheme overlap in these regions; in the yellow sector, their absorptions are of comparable strength, while in the orange, the third pigment is distinctly the more effective. As a consequence of this, the complementary colour to yellow would be at the violet end of the spectrum; as we move into the orange, the complementary colour would shift into the blue. A further shift towards the red would result in the complementary colour being located at the boundary between the green and blue sectors in the spectrum. The remarkable fact of observation that in a whole series of cases the superposition of only two monochromatic radiations with appropriate intensities results in a complete suppression of colour thus finds a simple and satisfactory elucidation on the basis of the present approach to colour theory.

4. SUPERPOSITION OF THE CHROMATIC AND ACHROMATIC SENSATIONS

We have seen that in certain cases, non-homogeneous light excites chromatic sensations identical with the colours of the spectrum or the purples derived therefrom, while in other cases the resulting sensation is achromatic. We may therefore assume that, in general, both of these effects would be manifested but to different extents depending on the particular circumstance of each case. In other words, the sensation excited by non-homogeneous light could, in general, be described as a superposition of the chromatic and achromatic sensations. The colours of the spectrum and the purples accordingly set an upper limit to the visual manifestations of colour. We infer that non-homogeneous light exhibits a third attribute besides luminosity and colour, namely, the purity or degree of saturation of the colour. The highest purity is that of the pure spectral colours and the purples derived therefrom, while the lowest purity represents the case

in which the achromatic part is relatively so large that no colour is discernible. Hand in hand with the concept of purity enters also the concept of dominant wavelength, which is the particular wavelength in the spectrum the colour of which the composite radiation under study most nearly resembles.

An interesting question arises here. Should the chromatic and achromatic sensations associated with non-homogeneous light be regarded as distinct effects or as inseparable from each other? If one thinks in physical terms, there is clearly a fundamental difference between them. An achromatic sensation would correspond to a chaotic and characterless disturbance; on the other hand, a pure spectral colour is associated with specifiable quanta of radiational energy. There is no reason why sensations so different in their nature and origin should be placed in the same category. It seems more appropriate to regard them as quite distinct attributes of the sensations excited by non-homogeneous light.

The very interesting results obtained by E.P.T. Tyndall and by G. Haase in their studies on colour discrimination with admixtures of monochromatic and white light have a bearing on the issue raised above. Measurements were made by these authors of the smallest change in wavelength of monochromatic light necessary to produce a detectable change of colour. The determinations were then repeated when white light was added in equal amounts to the two monochromatic fields of slightly different wavelength under comparison, the purity or degree of saturation of the colour in these fields being thus varied in different observations over a wide range. The remarkable result emerged that the chromatic sensibility of the eye to wavelength differences is not significantly diminished even when the white light added represents a fifty per cent. dilution of the visible colour. A result of this nature could scarcely have been anticipated unless the chromatic and achromatic sensations are distinct and unrelated effects.

6. The Results of Colour-Mixing Experiments

The simplest kind of experiment that could be made on the mixing of colours is to have only two monochromatic radiations, the spectral position and relative intensities of which could be varied, and to compare the sensation resulting from their superposition with another monochromatic radiation appearing in an intermediate position in the spectrum, the intensity of which can also be varied. The results of such comparison can be broadly indicated in the light of the remarks made above.

If both the selected radiations lie within the wavelength range between 530 mμ and 780 mμ, there would be little difficulty in obtaining a perfect

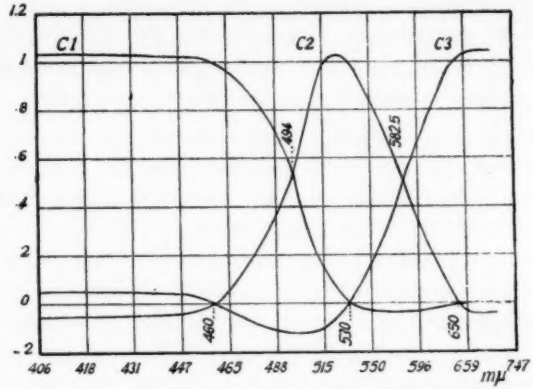

FIG. 1. Results of mixing 460 mμ, 530 mμ and 650 mμ.

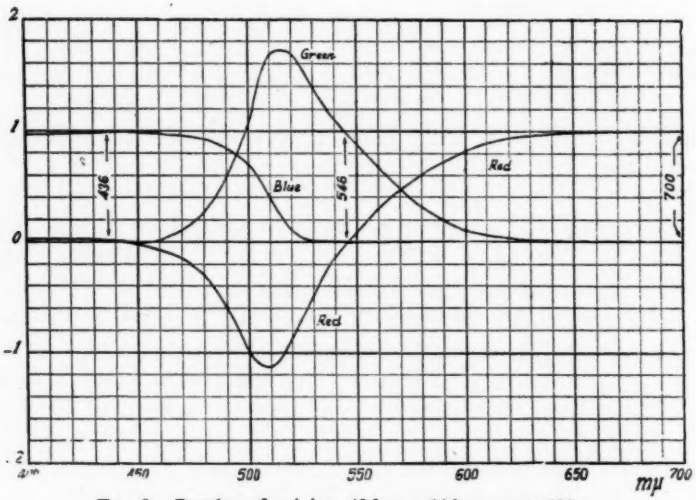

FIG. 2. Results of mixing 436 mμ, 546 mμ and 700 mμ.

match. Likewise, if one of the selected radiations is near the extreme red end or near the extreme violet end, and the other also lies in the violet or red sector of the spectrum as the case may be, there should be no difficulty in matching the result with some intermediate radiation. The situation would however be different if one of the selected radiations lies in the wave length range between 400 mμ and 530 mμ and the other also lies in that range, but not in an adjacent position. Only when the two selected radiations are quite close to each other that it would be possible to obtain a good

match. The further away they are, the less and less satisfactory would be the result, until finally when the two are sufficiently far apart, there could be no comparison at all. The position would be far worse if one of the radiations is in the wavelength range between 400 mμ and 530 mμ and the other is in the range between 530 mμ and 750 mμ. We would then be approaching a situation in which the result of mixing the two monochromatic colours would be to obtain an achromatic sensation.

Figure 1 and Fig. 2 above represent the results of experiments of the same nature as that indicated above with the difference that three instead of two monochromatic radiations were chosen and employed and while their positions in the spectrum were kept fixed, their intensities were varied with a view to obtain a match with the spectral colours appearing over the whole range of the spectrum. In Fig. 1, the three chosen wavelengths were 460 mμ, 530 mμ and 650 mμ and the results represented are those of W. D. Wright and collaborators. In Fig. 2, the chosen wavelengths were 436 mμ, 546 mμ and 700 mμ, the two former being the strong lines in the mercury arc spectrum. The graphs appearing in the figure represent the values of the coefficients C_1 (blue), C_2 (green) and C_3 (red) which indicate the quantities of blue, green and red light necessary to obtain the match represented by the colour equation

$$C_1B + C_2G + C_3R = \text{Chosen spectral colour},$$

where

$$C_1 + C_2 + C_3 = 1.$$

Figures 1 and 2 show certain features in common and also some noteworthy differences. We shall first mention the former and remark on their significance in relation to the absorptive properties of the visual pigments. In both figures, the coefficient C_1 (blue) has a value of nearly unity in the violet sector of the spectrum and then drops down steeply in the wavelength range 480 mμ to 530 mμ and is negligible or zero at all wavelengths greater than 530 mμ. The behaviour of C_1 thus clearly follows the absorption characteristics of xanthophyll. Then again, in both figures, the graphs for C_2 (green) and C_3 (red) overlap in the wavelength region between 550 mμ and 625 mμ; C_2 diminishes and C_3 increases in this range, the graphs crossing at 582 mμ in Fig. 1 and at 570 mμ in Fig. 2. C_2 becomes negligible in comparison with C_3 at all wavelengths greater than 625 mμ, while C_3 is dominant and practically unity in that region. Here, again, the behaviours of C_2 and C_3 recall the remarks made earlier regarding the overlapping of the absorption spectra of ferroheme and of ferriheme and its consequences.

The appearance of negative coefficients for C_3 in the spectral region between 460 mμ and 530 mμ is a well-marked feature in both figures but far more so in Fig. 2 than in Fig. 1, evidently because the blue and green radiations superposed were farther apart in the spectrum in the case of Fig. 2 than of Fig. 1. The appearance of these negative coefficients indicates that the superposition of the two monochromatic radiations results in a strong achromatic component in the sensation. A good measure of the third component has therefore to be added to the spectral colour under study to obtain a colour match. The production of an achromatic sensation by the superposition of monochromatic radiations in certain circumstances is thus an important and indeed basic feature in colour theory. The circumstances in which the achromatic sensation appears have already been discussed in Section 3 above and need not therefore be repeated here.

7. GEOMETRIC REPRESENTATIONS OF COLOUR

Figure 3 below reproduces the so-called XYZ chromaticity diagram. This represents in geometric form certain empirically determined colour relationships which have been put into a shape convenient for practical use.

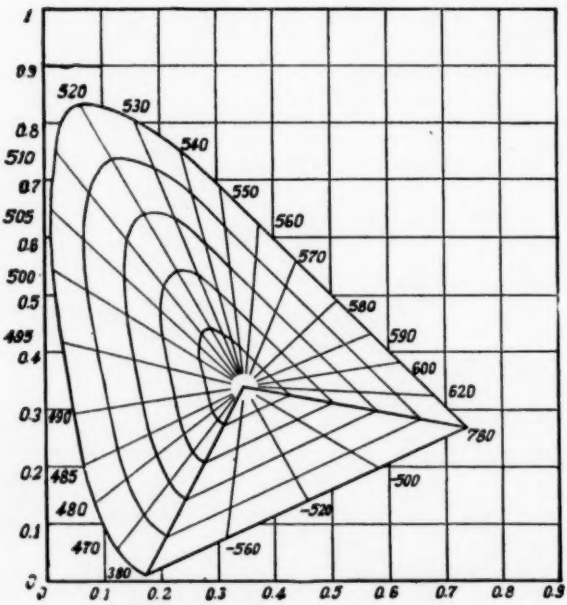

FIG. 3. The XYZ chromaticity diagram.

The diagram is reproduced here for the reason that the facts concerning colour vision elucidated in the preceding pages are evident on a simple inspection of it.

1. All observable colours appear as points inside a closed figure at the periphery of which appear the colours of the spectrum and the line of purples. The latter is a straight line joining the violet and red ends of the spectrum.

2. The spectral colours in the range between 530 mμ and 780 mμ appear on a line which is straight except very near 530 mμ where it exhibits a slight curvature.

3. Chromatic sensations complementary to each other are indicated by the two points on the periphery of the figure the straight line joining which passes through its white centre.

4. The degree of saturation or purity of any observed colour is indicated by its position in the figure on the line which joins the white centre with the point on the periphery representing the dominant wavelength.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part VI. Defective Colour Vision

By Sir C. V. Raman

(*Memoir No. 125 of the Raman Research Institute, Bangalore-6*)

Received December 1, 1960

1. Introduction

The subject of defects in colour vision is one of very general interest. The actual nature of the defects, the different types of defect and the relationships between them, the hereditary character of the defects, the frequency of their appearance in the human population and their distribution as between the sexes, the manner in which they can be brought to light and their relationship to occupational fitness are some of its different aspects. The scientific investigation of the characters of defective colour vision is a necessary preliminary to a deeper understanding of its nature and origin. In view of the many-sided nature of the subject, it has naturally been the theme of numerous studies and researches and an extensive literature has grown up dealing with it from different points of view.

In the present memoir, we shall concern ourselves chiefly with the fundamental aspects of defective colour vision. The subject will be dealt with on the basis of the ideas set forth and developed in the preceding parts of the memoir.

2. The Origin of the Defects

The defects of colour vision which we shall proceed to discuss in detail are of congenital origin and belong to the so-called sex-linked and recessive type of inheritable characters. The principles of genetics indicate, in agreement with observation, that defective colour vision should be far less common amongst women than amongst men; usually the woman acts simply as a carrier and transmits the defect without showing any characteristic anomaly herself. The fact that the defects are passed on from parents to progeny is of deep import and suggests that they stand in the closest relationship to the functioning of biochemical processes in the human body. In this connection, it is appropriate here to recall other inheritable characters

which are clearly of a biochemical nature. The blood group to which an individual belongs is one of them. Another is the rare condition known as hæmophilia, which is the failure of human blood to stop flowing out from a wound when a person has been accidentally injured. This is known to be a sex-linked recessive defect.

By far the commonest and most thoroughly studied defects in colour vision are those which relate to the perception of the light appearing in the region of the spectrum between 495 mμ and 780 mμ, in other words those covered by the green, yellow, orange and red sectors in the spectrum as perceived by a person with normal colour vision. In this region, as we have seen, vision is mediated by the two visual pigments ferroheme and ferriheme. These have similar structures which differ only in that the iron atom appearing at the central position of the tetrapyrollic group in one case is in the ferrous and in the other in the ferric state. The question naturally arises as to the nature of the biochemical mechanism which regulates the proportion in which the two pigments appear in the retina. The passage from the ferrous to the ferric state is a change in valency and may be regarded as an oxidative process. It is to be presumed that there is a mechanism at work by which the formation of the ferric pigment is permitted up to a certain proportion and its further progress beyond that point is inhibited.

If the suggestion made above represents the actual position, it follows that the mechanism might, at least in some cases, not function in the normal manner. For example, it is possible that the oxidation is totally inhibited, in which case only ferroheme would be present in the retina. Then again, there might be cases intermediate between such a complete inhibition and the normal functioning of the mechanism. It is also possible that there might be a lack of balance in the opposite direction and some cases in which the oxidation goes so far that ferriheme is in excess of that needed for normal colour vision.

In what follows, we shall consider the consequences of the biochemical situations indicated above and compare them with the actual facts elicited by studies of the different types of defective colour vision.

3. Protanopic and Protanamolous Vision

The cases of defective colour perception which came first under notice and received the largest share of attention were naturally those of the extreme kind which revealed themselves without any special efforts being made to discover them. Subsequently, however, scientific studies showed that the incidence of the defects is larger than was suspected and that there is a

considerable variation in the actual magnitude and character of the defects. At the present time, if we leave aside some rarer types, the kinds of colour vision which have been recognized and investigated are five in number: I. Protanopic vision (1%). II. Protanamolous vision (1%). III. Normal vision (92%). IV. Deuteranamolous vision (5%). V. Deuteranopic vision (1%). The approximate percentages of the male human population belonging to these classes have been entered in round figures after each of them. The first and the fifth kinds are usually grouped together as dichromatism, while the second and fourth are commonly referred to as anamolous trichromatism, in other words as variants of normal vision.

We shall first consider protanopic vision. Its characters may be deduced from those of normal colour vision discussed in detail in the fourth part of this memoir. In the absence of ferriheme, the distribution of luminosity in the spectrum, the colour sequence observed and the form of the hue discrimination curve would all be necessarily disturbed. The luminosity would be zero in the sector of the spectrum between 650 mμ and 780 mμ and would be diminished relatively to the rest in the regions of the spectrum where ferriheme, when present, contributes to the observed luminosity. The red sector would disappear completely from the spectrum. In normal vision, ferroheme and ferriheme co-operate in the regions where yellow and orange are seen, and hence in the absence of ferriheme these colours cannot be perceived. Protanopic vision which operates by the mediation of only two pigments, namely, xanthophyll and ferroheme, would accordingly present only two sectors in the spectrum, one on either side of the wavelength 495 mμ which forms the boundary between blue and green in the normal colour sequence. Hence, as in normal vision, that wavelength would continue to be the point in the spectrum where the colour as perceived by the protanope changes most rapidly. The features of the hue discrimination curve observed at greater wavelengths would, however, disappear and be replaced by a continuous falling off in the rate of change of observable colour with wavelength. The features of protanopic vision thus deduced are in agreement with those actually observed. The comparisons between normal and protanopic vision in respect of spectral luminosity and hue discrimination exhibited respectively in Figs. 1 and 2 are based on the observations of F.H.G. Pitt.

We now proceed to discuss the characteristics of protanamolous vision on the assumption that this type of vision results from a replacement of the ferroheme which alone is present in protanopic vision by a small proportion of ferriheme. The effect of such replacement on vision would be principally

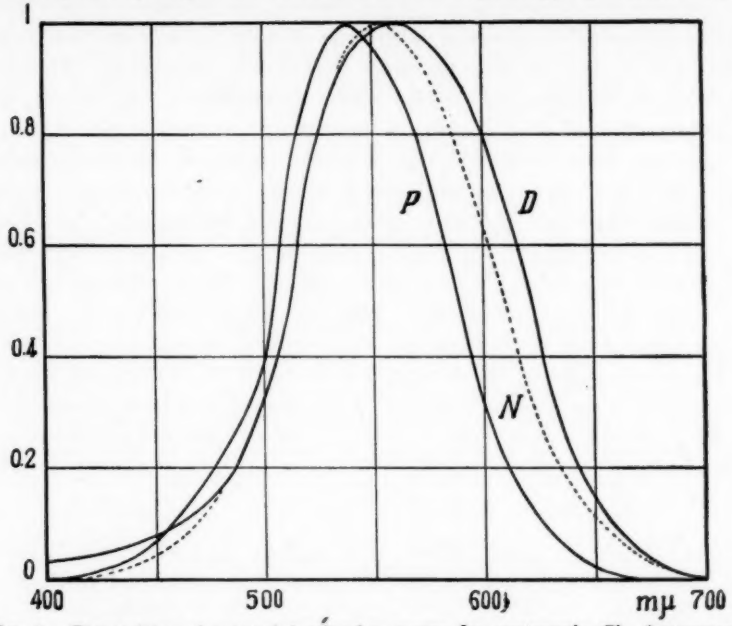

FIG. 1. Comparison of spectral luminosity curves for protanopic (P), deuteranopic (D) and normal vision (N).

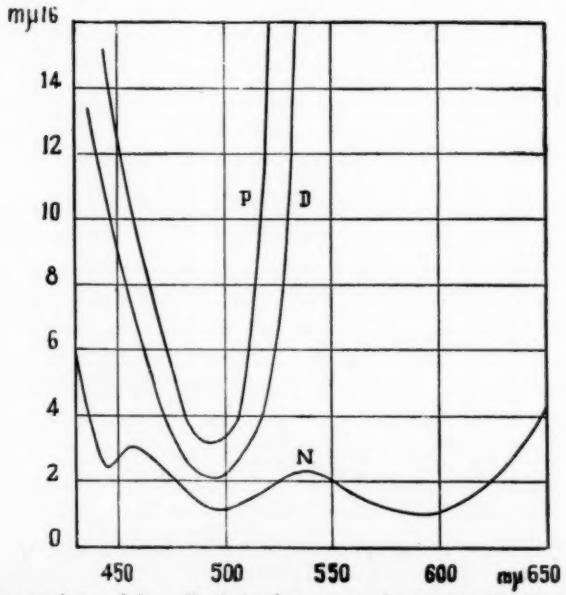

FIG. 2. Comparison of hue discrimination curves for protanopic (P), deuteranopic (D) and normal vision (N).

felt in the region of wavelengths where the absorption of ferriheme is comparable with or actually stronger than that of the ferroheme. This is the region between 560 mμ and 650 mμ where in normal colour vision, green changes over to yellow and then to orange and red. In this region, the protanamolous observer would see hues not perceived by the protanope, and recognise that they alter with the location in the spectrum. In other words, besides the rapid change in colour around 495 mμ which is apparent alike to the normal observer and the protanope, the protanamolous observer would observe colour changes roughly analogous to those apparent to normal vision in the region of wavelengths between 560 mμ and 650 mμ, but of a much less precisely defined character. The greater the quantity of ferriheme which replaces ferroheme, the more clearly would these features be perceived. It may therefore be expected that the protanamolous vision would exhibit in its hue discrimination curves a wide range of variation, approximating to normal colour vision at one end of the range to that of protanopic vision at the other end.

The conclusions reached above find support in the experimental data represented in Fig. 3 below of the hue discrimination curves of four pro-

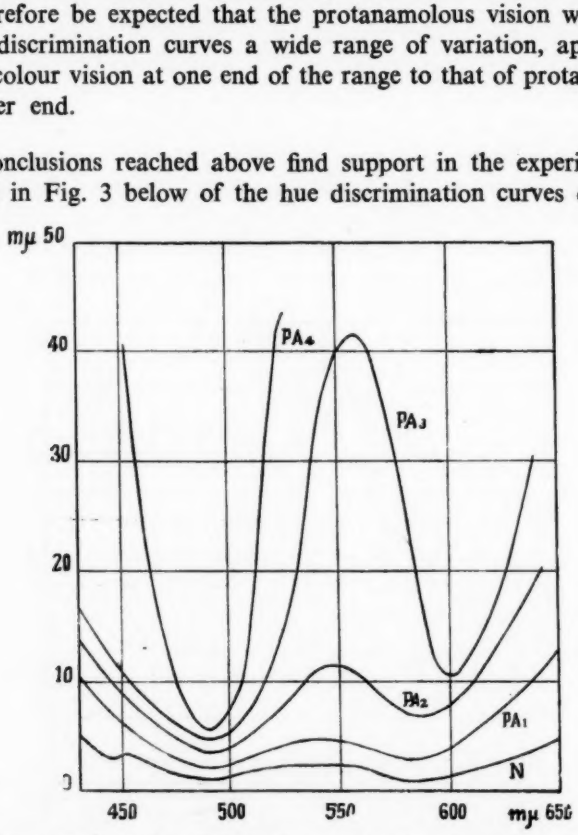

FIG. 3. Comparison of hue discrimination curves of protanamolous (Pa) and normal observers (N).

tanamolous observers and one normal observer which have been selected from the extensive set of data presented in a paper by McKeon and Wright.

4. Deuteranopic and Deuteranamolous Vision

A biochemical situation which results in the presence of an excess of ferriheme over ferroheme would have consequences in respect of colour vision which can readily be foreseen. As the ferroheme-ferriheme ratio diminishes, the region of wavelengths in the spectrum within which the absorptions of the two pigments are of comparable strength would shift towards shorter wavelengths and their overlap would extend and become effective until it completely covers the sector of the spectrum which appears green to a normal observer. In other words, the green and red sectors of the spectrum cease to have a separate existence and merge to form a tract in which the yellow and orange regions which ordinarily form only a fringe between them extend and cover the entire range of wavelengths referred to. Thus, instead of the normal colour sequence of green, yellow, orange and red, a band of colour would appear which may be described as yellow with a greenish tinge at one end and a reddish tinge at the other. This would cover the entire region where the absorption spectra of ferroheme and ferriheme co-operate. It would terminate at about 495 mμ where the blue-violet sector of the spectrum begins. Thus, again, as in protanopic vision, the spectrum consists of only two sectors meeting at about that wavelength. The difference between deuteranopic and protanopic vision is that in the former case, the spectrum goes up to the extreme red end instead of stopping off at shorter wavelengths. The colours observed would also be different in the two cases.

Observational evidence confirming the correctness of the foregoing explanation of deuteranopic colour vision is furnished by the spectral luminosity curves and the hue-discrimination data for several deuteranopic subjects which have been made available by the work of F. G. H. Pitt. These have been represented alongside of those of protanopic and normal observers in Figs. 1 and 2 above respectively in the text. They show very clearly the much greater extension of the spectral luminosity curves towards the red in deuteranopic as compared with protanopic vision. The difference between deuteranopic and normal vision in respect of the spectral luminosity curves is also distinctly shown. The hue discrimination curves, on the other hand, show a general similarity between protanopic and deuteranopic vision and a striking dissimilarity with the normal vision. The observations, however, reveal the noteworthy and significant fact that deuteranopic vision

has a distinctly better hue discrimination than protanopic vision in the wavelength range between 400 mμ and 530 mμ. But hue discrimination is completely lacking in the region of greater wavelengths in both types of vision.

We shall not here pause to discuss the results of colour mixing experiments made with observers having one or the other of these two types of defective vision, as also studies of the colour confusions which they exhibit when presented with test objects which appear of different hue to normal observers. These features of protanopic and deuteranopic vision are readily deducible from the fundamental results set forth above. We shall proceed to consider the features of deuteranamolous vision which are of special interest if only for the reason that this type of defective vision outnumbers all the other types put together. Deuteranamolous vision can be distinguished from normal vision by the method of observation by which its existence as well as that of protanamolous vision was discovered, *viz.*, that of requiring the person tested to match a monochromatic yellow by a mixture of monochromatic green and red radiations by varying their relative intensities. A protanamolous observer would require a smaller ratio of green to red than is required for normal vision, while a deuteranamolous observer would need a larger ratio. This is a clear indication that red light appears dimmer in protanamolous than in normal vision, while it appears brighter in deuteranamolous vision. Since protanomaly is explicable as a consequence of the ferroheme-ferriheme ratio being larger than the normal, it may be inferred that deuteranomaly is a result of ferriheme being present in the retina in excess of that required for normal colour vision.

The observable consequences of ferriheme being present in a proportion greater than normal should be of three kinds: (*a*) a shift of the spectral luminosity curves towards longer wavelengths as compared with normal; (*b*) a reduction in the power of hue discrimination at all wavelengths greater than 5000 Å and (*c*) changes of the chromatic coefficients, *viz.*, the intensity ratio between green and red monochromatic radiations of chosen wavelengths which when superposed would match the colours of the spectrum at various wavelengths. In respect of all these features, it may be expected that the magnitude of the observed changes would depend upon the actual proportion of ferriheme present. The more nearly the ferroheme-ferriheme ratio approaches normal, the greater would be the resemblance between deuteranamolous and normal vision. *Per contra*, the greater the excess of ferriheme present, the more would deuteranamolous vision tend to approach deuteranopic vision in its characters.

The observational evidence available supports the foregoing inferences. D. B. Judd in a published report has drawn the individual spectral luminosity

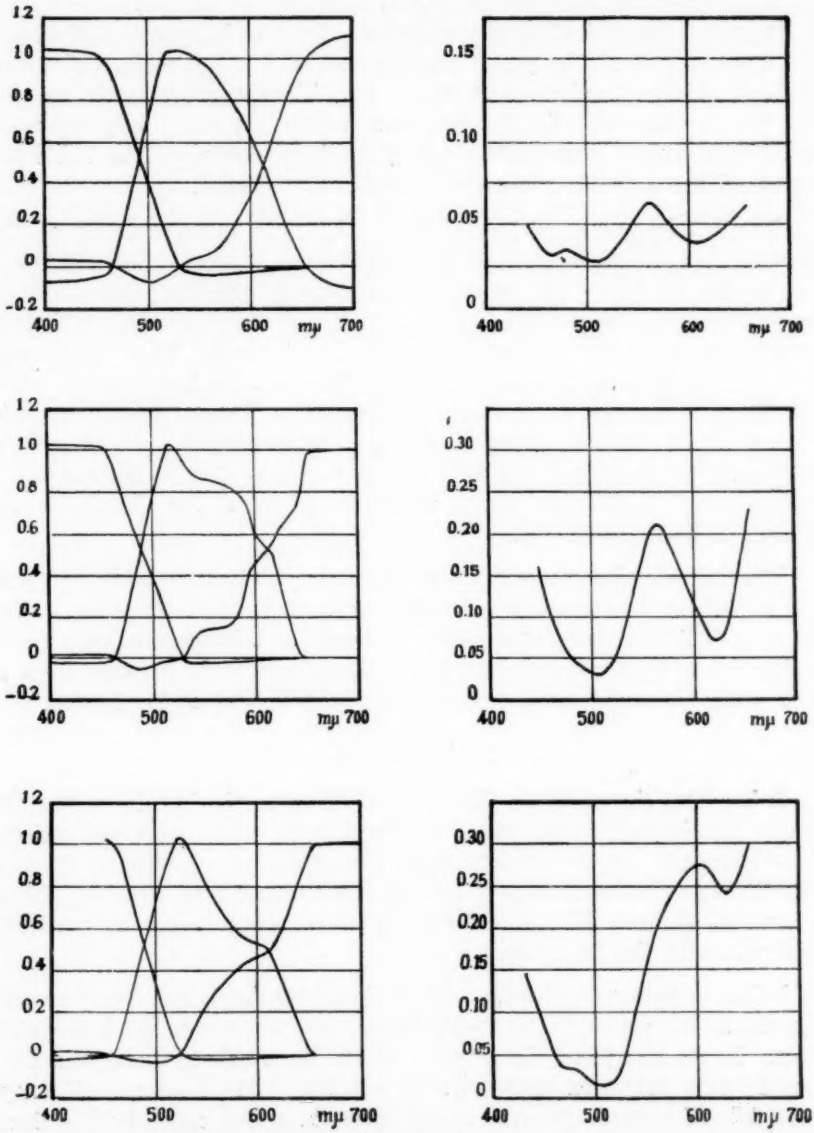

FIG. 4. (*a*) Spectral chromaticity coefficients and (*b*) hue discrimination curves of three deuteranamolous observers according to J. H. Nelson. The unit of the ordinates in Fig. 4 (*b*) is 100 mμ.

curves of twelve protanamolous and of six deuteranamolous observers and compared them respectively with the averages of the luminosity curves of six protanopes and of six deuteranopes. As is to be expected, the luminosity curves of both the protanamolous and of deuteranamolous observers exhibit a certain spread amongst themselves. The significant result emerges that the average curve for the six deuteranopes appears clearly separated and shifted towards longer wavelengths from the region where the individual curves of the deuteranamolous observers are recorded.

The fall in power of hue discrimination and the alterations of the chromatic coefficients are necessarily related to each other. For, the extension of the overlap of the absorption spectra of ferroheme and ferriheme due to the latter being present in excess would obliterate the colour difference between the green and the red sectors of the spectrum. This, on the one hand, would result in a large increase in the limen for hue discrimination and also shift the wavelength where the minimum of limen appears towards the red where there is no such overlap. Simultaneously, the chromatic coefficients for green and red would tend to approach and become equal to each other in the same region where the power of hue discrimination registers a large diminution.

The foregoing inferences find very clear support in the data represented in Fig. 4 where the chromatic coefficients and the hue discrimination curves for three different deuteranamolous observers are shown side by side to exhibit the correlations between them. These have been selected from a more extensive set of observational data published by J. H. Nelson.

5. TRITANOPIA

It has been shown in the third part of this memoir that the carotenoid pigment xanthophyll is the visual receptor in the blue and violet regions of the spectrum. Its absorption which is negligible at wavelengths greater than 520 mμ, rises very steeply around 490 mμ and after reaching fairly high values between 480 mμ and 440 mμ drops down again to relatively small values in the far violet.

It follows from what has been stated that if xanthophyll is totally absent from the retina, colour vision would be seriously affected in the blue-violet sector. In the first place, the observer would be unable to distinguish between the blue and green colours in the spectrum. The luminous efficiency would fall off towards the end of the spectrum rather more rapidly than it would for a normal observer. The hue discrimination curve should also show striking abnormalities. While the usual well-marked minimum of

the limen around 590 mμ would continue to be observed, the form of the curve in the green and blue regions would be totally different. The maximum value of the limen observed around 540 mμ and the minimum around 490 mμ would both disappear. Instead, there should be a progressive increase in the limen with diminishing wavelength at all wavelengths less than 590 mμ.

Though tritanopia is usually regarded as a rare condition, a search initiated by W. D. Wright involving the extensive publication of test colour charts resulted in a fairly large number of cases being discovered and investigated. The incidence of tritanopia has been found to be of the order of 1 in 20,000 persons. The indications are that tritanopia is an inherited condition but its incidence amongst women and men is not so widely different as in the cases of the more commonly observed types of defective vision. The consequences of the absence of xanthophyll in the retina indicated above are in general agreement with the findings of W. D. Wright. It was, however, noticed that while the power of hue discrimination virtually disappears in the blue-green wavelengths, it shows a rather surprising recovery near the far-violet end of the spectrum. This would seem to suggest that in the spectral region where the absorption of xanthophyll becomes very small, the other visual pigments ferroheme and ferriheme, play a not wholly negligible part in colour vision and colour discrimination.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part VII. General Summary

By Sir C. V. Raman

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1. Observations of the Retina

A simple but extremely powerful technique has been devised which enables an observer without any instrumental aid to see his own retina and study its functioning in varied circumstances. The observations made by this method have enabled important conclusions to be arrived at regarding the constitution of the retina and the mechanism by which light and colour are perceived. The observer sits facing a brightly lit white screen and views it through an appropriate colour filter held in front of his eye. After a sufficient interval of time, he fixes his vision on some particular point on the screen and then removes the filter. An enormously magnified picture of the retina then appears on the screen, the nature of which depends very much on the particular colour filter used. The explanation of the phenomenon is that the rays of the spectrum which in the first instance are absorbed by the filter, suddenly impinge on the retina when the filter is removed, and excite localised sensations over its different areas. These sensations project themselves on the observing screen as an enlarged image of the retina.

By correlating the absorption spectra of the filters used with the pictures of the retina perceived by the observer, it has been ascertained that the retina contains three visual pigments whose absorption spectra lie in different regions of the spectrum: Pigment A has an absorption lying in the spectral range 4000 to 5000 Å. Pigment B exerts an extremely powerful absorption in the wavelength range between 5000 and 6000 Å, while pigment C exerts a moderately powerful absorption in the region from 6000 to 7000 Å, but its absorption also extends into and partly overlaps the region covered by pigment B.

2. The Role of the Visual Pigments

The nature and properties of light when correlated with the observed facts concerning the perception of light and colour enable us to determine

the role played by the visual pigments in the retina. Each different monochromatic light in the spectrum represents radiation characterised by a different energy-quantum; the magnitude of the quantum increases progressively from the red to the violet end of the spectrum. It is a fact also that the colours observed in the spectrum change progressively and continuously from one end of it to the other. Some 250 different colours can be perceived over the whole range. A change of only one half of one per cent. in the magnitude of the light-quantum incident on and absorbed by the retinal pigments is usually sufficient to make an observable change of perceived colour, while in some parts of the spectrum a much smaller difference is thus detectable.

Monochromatic radiation is the fundamental entity with which the physicist is concerned in optics and spectroscopy. It follows from what has been stated that the fundamental visual sensations are also those excited by monochromatic radiation. The observed precision of colour perception would be inexplicable except on the hypothesis that the function of the visual pigments in the retina is to receive, absorb and then to pass on the absorbed energy-quanta to the central parts of the visual mechanism without any addition or subtraction, themselves returning to their original energy-states.

3. Identification of the Visual Pigments

The identification of the visual pigments presents no particular difficulties. In day-light vision, there is a highly pronounced maximum of luminosity in the spectrum at about 5600 Å, on either side of which the brightness falls off rapidly. It follows that pigment B of which the absorption lies in the green plays the major role in human vision, a role somewhat analogous to that which the absorption in the red by chlorophyll plays in the photosynthesis by green leaves. We can, therefore, unhesitatingly identify our pigment B with ferroheme which exhibits a powerful absorption of light in the green sector located at the same position as the maximum of visual luminosity in the spectrum.

Oxidation-reduction mechanisms play a fundamental role in the chemistry of the living structures of aerobic organisms. The recognition of ferroheme as the principal visual pigment thus automatically involves the identification of pigment C which appears in the retina in close association with pigment B as ferriheme. It is known that the absorption of light by ferriheme is weaker than that of ferroheme, but extends much further towards longer wavelengths and is indeed sensible up to the extreme red and of the spectrum. These are the properties needed for the visual pigment which functions in that region.

The pigment A which absorbs light between 4000 and 5000 Å and is the mediator of vision in the blue and violet sectors of the spectrum can be

none other than the carotenoid pigment xanthophyll which gives the characteristic golden yellow colour to the yoke of the common hen's egg. Xanthophyll finds its way into the human body through the consumption of food products containing it, and its presence in the retina is therefore not a matter for surprise. Indeed, it is the same yellow pigment which led to the anatomical name of *macula lutea* being given to the physiologically most important area in the retina.

4. COLOUR AND LUMINOSITY IN THE SPECTRUM

The known features of the absorption spectra of the visual pigments enable a satisfactory explanation to be given of the distinctive features noticeable in the different sectors of the spectrum. The very steep rise in the absorption by xanthophyll which appears in the wavelength range around 4900 Å is responsible for the rapid change in the colour of the spectrum from green to blue and the very low value of the limen of wavelength alteration needed for observable change of colour appearing in that region. The similar but less striking fall of the limen in the region around 4400 Å where the colour of the spectrum changes from blue to violet is likewise attributable to the rapid fall of the absorption by xanthophyll with diminishing wavelength appearing in that region.

The overlap in the absorption spectra of ferroheme and ferriheme between 625 mμ and 566 mμ gives rise to the appearance of yellow and orange in the spectrum as an interpolation between the green and red sectors, the yellow where the absorptions of the two pigments are of comparable strength, and the orange where the absorption by ferriheme is stronger than by ferroheme. The steep fall in the absorption by ferroheme in the same region is responsible for the limen of wavelength change needed for an observable difference of colour reaching very low values in the region around 590 mμ.

The progressive fall in the luminosity of the spectrum and the increasing limen of wavelength change for a perceptible colour difference manifested near the extreme violet and red ends of the spectrum appear as consequences respectively of the diminishing absorption and the diminishing slope of the absorption curves of xanthophyll and of ferriheme in those two regions.

Some remarkable effects are observed when a colour filter which transmits only the blue-violet part of the spectrum is held in front of the eye and a polaroid is placed and alternately taken out and put in before the filter. These effects cease to be observable when the illumination of the field under observation is diminished so as to fall below the photopic level. They afford

a conclusive demonstration that xanthophyll is the visual pigment which enables us to perceive the blue and violet colours of the spectrum.

5. Non-Homogeneous Light

The visual sensations produced by heterogeneous light result from the synthesis by the centre of the sensations excited by the monochromatic radiations of which it is composed. The results of the synthesis may be either a chromatic or an achromatic sensation. Chromatic sensations arise when only two of the three visual pigments function, as for example when the radiations from the extreme red and violet ends of the spectrum are superposed, giving rise to the purples, or when the superposed radiations lie in the spectral range where xanthophyll has no absorption, *viz.*, in the red and green sectors; the resultant sensation is then a pure spectral colour.

The achromatic sensation arises when all the three visual pigments function in appropriate strengths. This enables a satisfactory explanation to be given of the fact that in a whole series of cases, the superposition of only two monochromatic radiations of appropriately chosen wavelengths results in the complete abolition of colour. In general, the sensations excited by heterogeneous light are a mixture of the chromatic and achromatic sensations which may be regarded as independent effects. The fact that all observable colours may be regarded as a superposition in appropriate proportions of white light and a pure spectral colour (including the pure purples) thus receives a satisfactory explanation.

The results obtained in experiments in which a pure spectral colour is sought to be reproduced by the superposition of other spectral colours may be interpreted in the same manner. The so-called spectral chromaticity coefficients determined in such experiments, when they have positive values, exhibit a parallelism with the absorption strength of the visual pigments which function in the respective spectral regions. *Per contra*, the appearance of negative values of the coefficients indicates that achromatic sensations are produced.

6. Defective Colour Vision

The existence of both ferroheme and ferriheme as visual pigments in the retina presupposes that there is a biochemical mechanism which determines the proportions in which they are normally present. Any deviations of the mechanism from normality would result in ferriheme being either totally absent or else being present much in excess. There would also be intermediate cases. The existence of four types of inheritable defect in colour

vision would thus be explicable in terms of the biochemical mechanism which determines the ferroheme-ferriheme ratio in the retina. These are respectively the protanopic, protanomalous, deuteranomalous and deuteranopic types of colour vision. The features of these different types of defects are readily predictable and the results thus deduced are in agreement with the observed facts. Tritanopia is likewise explicable as due to the complete absence of xanthophyll from the retina.

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